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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

ALCOHOL RESEARCH Current Reviews

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Alcohol Use Disorder and Dementia: A Review

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PURPOSE: By 2040, 21.6% of Americans will be over age 65, and the population of those older than age 85 is estimated to reach 14.4 million. Although not causative, older age is a risk factor for dementia: every 5 years beyond age 65, the risk doubles; approximately one-third of those older than age 85 are diagnosed with dementia. As current alcohol consumption among older adults is significantly higher compared to previous generations, a pressing question is whether drinking alcohol increases the risk for Alzheimer's disease or other forms of dementia.
SEARCH METHODS: Databases explored included PubMed, Web of Science, and ScienceDirect. To accomplish this narrative review on the effects of alcohol consumption on dementia risk, the literature covered included clinical diagnoses, epidemiology, neuropsychology, postmortem pathology, neuroimaging and other biomarkers, and translational studies. Searches conducted between January 12 and August 1, 2023, included the following terms and combinations: "aging," "alcoholism," "alcohol use disorder (AUD)," "brain," "CNS," "dementia," "Wernicke," "Korsakoff," "Alzheimer," "vascular," "frontotemporal," "Lewy body," "clinical," "diagnosis," "epidemiology," "pathology," "autopsy," "postmortem," "histology," "cognitive," "motor," "neuropsychological," "magnetic resonance," "imaging," "PET," "ligand," "degeneration," "atrophy," "translational," "rodent," "rat," "mouse," "model," "amyloid," "neurofibrillary tangles," "α-synuclein," or "presenilin." When relevant, "species" (i.e., "humans" or "other animals") was selected as an additional filter. Review articles were avoided when possible.
SEARCH RESULTS: The two terms "alcoholism" and "aging" retrieved about 1,350 papers; adding phrases—for example, "postmortem" or "magnetic resonance"—limited the number to fewer than 100 papers. Using the traditional term, "alcoholism" with "dementia" resulted in 876 citations, but using the currently accepted term "alcohol use disorder (AUD)" with "dementia" produced only 87 papers. Similarly, whereas the terms "Alzheimer's" and "alcoholism" yielded 318 results, "Alzheimer's" and "alcohol use disorder (AUD)" returned only 40 citations. As pertinent postmortem pathology papers were published in the 1950s and recent animal models of Alzheimer's disease were created in the early 2000s, articles referenced span the years 1957 to 2024. In total, more than 5,000 articles were considered; about 400 are herein referenced.
DISCUSSION AND CONCLUSIONS: Chronic alcohol misuse accelerates brain aging and contributes to cognitive impairments, including those in the mnemonic domain. The consensus among studies from multiple disciplines, however, is that alcohol misuse can increase the risk for dementia, but not necessarily Alzheimer's disease. Key issues to consider include the reversibility of brain damage following abstinence from chronic alcohol misuse compared to the degenerative and progressive course of Alzheimer's disease, and the characteristic presence of protein inclusions in the brains of people with Alzheimer's disease, which are absent in the brains of those with AUD.

KEYWORDS: alcohol; aging; Alzheimer disease; neuropsychology; neuropathology; magnetic resonance imaging; positron-emission tomography; rodent

In 2020, an estimated 17% of the U.S. population was older than age 65; this proportion is projected to rise to about 23% by 2060.[1,2] This prompts an urgent need for identifying potential and modifiable risk factors contributing to health decline.[3,4] After tobacco, alcohol is the most misused substance in the United States and abroad.[5] Even prior to the coronavirus disease 2019 (COVID-19) pandemic, which contributed to increased drinking rates, alcohol consumption was notably accelerating in several demographic categories, including in men and women older than age 65.[6-8] Consuming alcohol in harmful patterns—such as binge drinking (five or more drinks in men, or four or more drinks in women, in about 2 hours; where a drink is equivalent to 12 oz beer, 5 oz wine, or 1.5 oz distilled spirits)—occurs in more than 25% of older Americans;[5,9] annual growth trends in alcohol misuse are reported to be 2.4% in older men and 1.6% in older women.[10]

Although not causative, older age is a risk factor for dementia: Every 5 years beyond age 65, the risk doubles;[11] and approximately one-third of people over age 85 are diagnosed with dementia.[12,13] Emerging data support alcohol misuse as a risk factor for dementia.[14] This review considers the literature to determine whether chronic alcohol misuse increases the risks for (1) alcohol-related dementias, including Wernicke-Korsakoff syndrome (WKS); (2) Alzheimer's disease; or (3) other forms of dementia (i.e., vascular, frontotemporal, or Lewy body dementia).

Search Methods and Results

Table 1 presents details regarding the literature searches conducted in preparation for this review. For each section in this article, search terms initially included a combination encompassing alcohol use (e.g., alcohol consumption, alcoholism, binge alcohol, alcohol abuse, alcohol use disorder) and cognitive impairment (e.g., dementia, WKS, Alzheimer's disease), which were then narrowed to the relevant topic (e.g., clinical diagnoses, epidemiology, neuropsychology). Several search terms describing alcohol use were used as the more traditional term "alcoholism" resulted in far more citation results than the currently accepted term, "alcohol use disorder (AUD)." For example, the combination of the traditional term "alcoholism" with "dementia" resulted in 876 citations, but using the currently accepted term "alcohol use disorder (AUD)" with "dementia" produced only 87 papers. Similarly, whereas the terms "Alzheimer's" and "alcoholism" yielded 318 results, "Alzheimer's" and "alcohol use disorder (AUD)" returned only 40 citations. The searches also considered subtypes of dementia in addition to Alzheimer's disease, such as alcohol-related WKS and vascular, frontotemporal, and Lewy body dementias. Searches regarding animal models (i.e., rat, mouse) were narrowed by pathological terms or relevant mechanisms (e.g., amyloid, neurofibrillary tangles, presenilin).

The two terms "alcoholism" and "aging" retrieved about 1,350 papers; adding phrases (for example, "postmortem" or "magnetic resonance") limited the number to fewer than 100 papers. As pertinent postmortem pathology papers were published in the 1950s and recent animal models of Alzheimer's disease were created in the early 2000s, articles referenced span the years 1957 to 2024. In total, more than 5,000 articles were considered; approximately 400 are referenced herein (i.e., only articles directly related to search terms were included).

Results of the Reviewed Studies

Human Studies

Clinical diagnoses

Diagnoses of psychiatric illnesses typically rely on use of one of two manuals: the *International Classification of Disease* (*ICD*) first published in 1984 by the World Health Organization (WHO; 11th edition [*ICD-11*] implemented in 2022); or the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*) first printed in 1952 by the American Psychiatric Association (fifth edition [*DSM-5*] released in 2013). *ICD* codes are commonly used by primary care physicians, whereas *DSM* codes are more often used by psychiatrists and psychologists. Complicating consistent diagnoses is the evolution over time of concepts underlying clinical diagnoses of alcohol misuse or dementias. Thus, publications have considered diagnosis rates by comparing criteria in *ICD* to *DSM*,[15-17] *ICD* versions,[18,19] *DSM-IV* to *DSM-5* AUD,[20-24] *ICD* AUD,[25] *ICD* neurocognitive disorders,[26] *DSM* neurocognitive disorders;[27] bias in AUD[28,29] and dementia[30-32] diagnoses has also been reviewed.

The diagnosis of an alcohol problem is best made by review of medical histories and interviews with patients. Laboratory tests have low sensitivity, and physical examinations are generally helpful only after the repercussions of alcohol misuse are apparent.[33-35] Consequently, *ICD* diagnoses of AUD in primary care settings typically depend on the presence of health-related conditions, including alcohol-related mental health diagnoses, alcohol-related physical health diagnoses, or evidence for medication prescribed to treat alcohol-related problems.[36] AUD diagnosed using *DSM-5* requires the patient to meet two of 11 criteria; however, specialists—including psychiatrists, psychologists, social workers, and licensed counselors—use *DSM* criteria for diagnosis with questionable consistency.[24] Despite extensive public health efforts by the National Institute on Alcohol Abuse and Alcoholism, the Centers for Disease Control and Prevention, and the U.S. Preventive Services Task Force, current estimates are that fewer than 50% of people who visit primary care providers for alcohol-related issues are asked about the problem. Alcohol screening with validated questionnaires—i.e., the 10-question Alcohol Use Disorders Identification Test

Table 1. Literature Search Details

Relation evaluated	Alcohol consumption and dementia	
Databases used	PubMed, Web of Science, and ScienceDirect	
Literature covered	Clinical diagnoses, epidemiological findings, neuroimaging, neuropsychological profiles, other biomarkers, postmortem pathology, and translational studies	
Literature search dates	January 12, 2023–August 1, 2023	
Literature search terms	"aging," "alcoholism," "alcohol use disorder (AUD)," "brain," "CNS," "dementia," "Wernicke," "Korsakoff," "Alzheimer," "vascular," "frontotemporal," "Lewy body," "clinical," "diagnosis," "epidemiology," "pathology," "autopsy," "postmortem," "histology," "cognitive," "motor," "neuropsychological," "magnetic resonance," "imaging," "PET," "ligand," "degeneration," "atrophy," "translational," "rodent," "rat," "mouse," "model," "amyloid," "neurofibrillary tangles," "α-synuclein," "presenilin"	
Additional filters	Species (i.e., "humans" or "other animals")	
Results*	1,339	"alcoholism" and "aging"
	876	"alcoholism" and "dementia"
	498	"alcohol consumption" and "dementia"
	318	"Alzheimer's" and "alcoholism"
	231	"Alzheimer's" and "alcohol consumption"
	87	"alcohol use disorder (AUD)" and "dementia"
	60	"alcoholism" and "aging" and "magnetic resonance"
	40	"Alzheimer's" and "alcohol use disorder (AUD)"
	31	"alcoholism" and "aging" and "postmortem"

*Source: PubMed, August 14, 2023.

(AUDIT), the 3-question AUDIT-C on consumption, or the 4-question CAGE (Cut down, Annoyed, Guilty, Eye opener)—occurs in only about 2.5% of primary care visits in the United States.[37-39] The Substance Abuse and Mental Health Services Administration (SAMHSA) is another source of alcohol use data based on self-report.[40] As with *ICD* and *DSM* diagnoses, recognized limitations of SAMHSA data include frequent methodological changes (e.g., definitions of alcohol misuse), which hamper longitudinal comparisons.[40] Irrespective of criteria used (i.e., *ICD*, *DSM*, self-report), AUD is underdiagnosed.[37,41,42] Henceforth in this review, "AUD" refers to diagnoses made via any version of *ICD* or *DSM* criteria; otherwise, levels and frequency of alcohol consumption are indicated.

"Dementia" is an umbrella term for a decline in mental (i.e., cognitive, intellectual) functioning that interferes with daily life but does not disturb consciousness or perception. More than 100 subtypes of dementia have been recognized, including proteinopathy (e.g., Alzheimer's, frontotemporal, Lewy body dementia), vascular (i.e., related to blood vessels), and toxic/metabolic (e.g., alcohol-related, WKS) dementias.[43,44] *ICD* added the code for Alzheimer's disease in 1975, and *DSM* added the diagnosis in 1983. Both *ICD-11* and *DSM-5* use the term "neurocognitive impairment" to encompass many types of dementia diagnoses. Diagnosing dementia is difficult owing to its insidious onset as well as the range and diversity of symptoms that can resemble normal aging.[45,46] Indeed, differential diagnoses are imprecise[47,48] as the clinical signs and symptoms of the many dementias are essentially the same;[49,50] criteria and nomenclature for dementia subtypes remain imperfect;[51-53] and selective and specific in vivo biomarkers are still in development.[54,55] Further, as formal dementia differential diagnoses with currently accepted criteria are resource-intensive, up to 85% of dementia diagnoses are made by non-specialist, primary care clinicians.[56]

Epidemiological findings

Patients who develop Alzheimer's disease may initially present with mild cognitive impairment (MCI), defined as a measurable age-accelerated decline in cognition.[57] Among patients with documented MCI, one-third progress to a diagnosis of Alzheimer's disease,[58] which requires the presence of autopsy-detected neuritic plaques and neurofibrillary tangles.[49,57,59] Alzheimer's disease is frequently diagnosed (50% to 75% of dementia cases), but the diagnosis is rarely validated with imaging (i.e., positron emission tomography [PET]) or postmortem examination.[60-63] When autopsies are conducted, between 12% and 23% of patients diagnosed antemortem with Alzheimer's disease do not show defining neuropathology, suggesting that current prevalence estimates of Alzheimer's disease are high.[64,65] Vascular dementia, the second most diagnosed subtype (up to 20% of cases), often coexists with and is incorrectly diagnosed as Alzheimer's disease.[66,67] The

remaining dementias are typically categorized as Lewy body, frontotemporal, or alcohol-related.[68]

Compared with other types, alcohol-related dementia tends to have an early onset (i.e., ages 45 to 64) and slow progress.[69-71] In addition to alcohol-related dementia, thiamine deficiency (i.e., Wernicke's encephalopathy) can occur in settings of high alcohol consumption and in malnutrition due to other causes (e.g., parenteral feeding, bariatric surgery, severe pregnancy-related vomiting).[72,73] The acute nutritional deficiency is reversible if adequately treated but can otherwise advance to WKS characterized by severe, persistent, cognitive impairment predominantly affecting memory.[74] In contrast to Alzheimer's disease, alcohol-related dementia and WKS are more commonly diagnosed in men than women[75-77] and are less likely to be identified as such for several reasons, including underreporting of the extent of alcohol consumption, diagnosis perception bias, and a lack of standardized measures of thiamine.[78,79]

Epidemiological studies support alcohol misuse and AUD as a risk factor for all types of dementia (i.e., collapsed across subtypes). For example, a study in France using *ICD-10* codes to define AUD (codes F10.1–F10.9, Z50.2, F10.20–F10.23) and dementia (codes F00–F03, F05.1, F1x.73, G30, G31, I67.3, R54) found that AUD was a major risk factor for all types, but especially early-onset dementia (before age 65).[77] A Danish cohort comparing people with *ICD-10*–diagnosed alcohol dependence (code F10.2) and dementia (codes F00–F03, G30) with controls matched on sex, date of birth, and municipality reported twice the hazard ratio for dementia among men and women with alcohol dependence.[80] A U.S. study of more than 4,000 women veterans over age 55 that used *ICD-9* codes to define AUD (codes 305.00, 305.01, 303.00, 303.01, 303.02, 303.90, 303.91, 303.92) and dementia (i.e., a comprehensive *ICD-9* code list provided by the Veterans Health Administration)[81] determined that dementia developed in 1.1% of women without AUD and in 3.7% of women with AUD.[60] The United Kingdom Whitehall II study—using alcohol consumption patterns derived from questionnaires and *ICD-10*–defined dementia (codes F00–F03, F05.1, G30, G31)—demonstrated that, compared with people who drank moderately (i.e., 1 to 14 alcohol units/week), those who drank heavily (i.e., more than 14 alcohol units/week) had increased risk for developing *ICD-10* dementia.[82] Similarly, an analysis of seven cohorts from the United Kingdom, France, Sweden, and Finland, using self-reported alcohol consumption metrics and *ICD-10* dementia (codes F00–F03, G30, G31, I20–I25, I61, I63–I66, I67.2, I67.3, I67.4, I67.8, I69.3), found that relative to people who drank moderately (i.e., 1 to 14 drinks/week), those who drank heavily (i.e., more than 14 drinks/week) had a 1.2-fold greater risk of developing dementia; and noted associations between high alcohol consumption and early onset dementia.[83]

With respect to the effects of alcohol misuse and AUD on subtypes of dementia, findings are equivocal. A U.S.-based study using data from commercially insured and Medicare Advantage beneficiaries suggested that AUD (*ICD-9* codes 291*, 303*, 305.0*, 357.5, 425.5, 535.3, 571.0, 571.1, 571.2, 571.3; *ICD-10* codes F10*, G31.2, G62.1, G72.1, I42.6, K29.2, K70*, K85.2, K86.0, Q86.0) specifically increased the risk for Alzheimer's disease (*ICD-9* code 331.0; *ICD-10* codes F00*, G30*).[84] A study using "driving under the influence" as a proxy for alcohol addiction reported that it was associated with an earlier "Alzheimer's disease" diagnosis; however, the *ICD-9* codes used in this study (i.e., 290.0–290.3, 290.8–290.9, 331.0) were not specific for Alzheimer's dementia.[85] A study using criteria-based diagnoses of dementia and chart-confirmed alcohol misuse (defined as "alcohol consumption that negatively impacts work or social life or leads to legal ramifications") demonstrated that alcohol misuse was a frequent presenting symptom of frontotemporal but not Alzheimer's dementia.[86] Other studies yielded inconclusive results regarding the relationship between alcohol consumption and frontotemporal dementia.[87,88] Moderate to heavy alcohol consumption (i.e., ≥ 7 drinks/week for women, ≥ 14 drinks/week for men) increased the risk for all types of stroke (i.e., ischemic and hemorrhagic stroke) and may thus be a risk factor for vascular dementia,[89-91] but results are inconsistent.[92,93]

In summary, alcohol misuse and AUD increase risk for all types of dementia. Assuming that 20% of AUD goes unrecognized and 20% of dementias are incorrectly classified as Alzheimer's disease, one might speculate that a significant proportion of dementia misclassification includes alcohol-related dementia. Reports that AUD specifically increases Alzheimer's disease likely overestimate the relationship.[94-96]

Neuropsychological profiles

A constellation of executive cognitive functions—including working memory, set shifting (i.e., the ability to unconsciously shift attention between tasks), problem-solving, and attention—are especially vulnerable to the effects of advancing age.[97-99] The neuropsychological profile of AUD uncomplicated by neurological confounders (e.g., WKS, hepatic encephalopathy) also includes deficits in executive functions.[100-102] Additionally, people with uncomplicated AUD show impairments in episodic memory (i.e., the ability to learn, store, and retrieve information about unique personal experiences including time and place),[103] visuospatial processing (i.e., the ability to perceive, analyze, and manipulate visual patterns and images, such as copying complex figures or orienting three-dimensional objects),[104,105] social cognition (i.e., the ability to interpret social information and behave appropriately),[106,107] and gait and balance.[108]

Features of WKS are persistent inability to remember new information (i.e., anterograde amnesia) and occasional confabulation.[74,109] Compared with non-alcohol-related WKS, the neuropsychological profile of alcohol-related WKS is broader and commonly includes executive dysfunction.[110-113]

Meta-analyses suggest that immediate and delayed memory tests (e.g., word-list recall) have high diagnostic accuracy in differentiating people with Alzheimer's disease from individuals

without the disease but poorly discriminate those with and without MCI.[114,115] Among available tools, the Montreal Cognitive Assessment (score ≤ 24), the Mini-Mental State Examination (MMSE, score ≤ 26), and the Dementia Rating Scale (score ≤ 124) appear to be efficient at discriminating MCI from aging without cognitive impairment.[116,117]

Refined neuropsychological data can help distinguish dementia subtypes. For example, people with Alzheimer's disease have more severe deficits in working and delayed memory than do those with WKS.[118-120] In people with AUD or Alzheimer's disease, the degree of impairment in verbal fluency, working memory, and frontal functions can be similar; memory problems, however, are more pronounced in Alzheimer's disease relative to AUD.[121] Similarly, although individuals with alcohol-related dementia or vascular dementia can show executive control deficits, they have less severe memory impairments than observed in those with Alzheimer's disease.[122] Further, patients with alcohol-related dementia demonstrate stabilization of functional impairment with abstinence, whereas those with Alzheimer's disease or vascular dementia show a progressive decline in cognitive functions.[123] Indeed, in a longitudinal study, people with alcohol-related dementia with monitored abstinence showed improved performance on executive functioning tests, whereas people with Alzheimer's disease performed worse on memory tests over the same time spans.[124] The amount of alcohol consumed was unrelated to cognitive performance in patients with *DSM-III*–defined "primary degenerative dementia."[125] In a more recent study of people diagnosed with MCI (*ICD-10* code F067) and evaluated by structured interview for alcohol use—i.e., low (less than 1 drink/week), moderate (1 to 14 drinks/week for men and 1 to 9 drinks/week for women), or heavy (more than 14 drinks/week for men and more than 9 drinks/week for women)—levels of alcohol consumed had no effect on MMSE scores; however, MMSE scores are notoriously insensitive to AUD-related cognitive decline.[126,127]

In summary, neuropsychological profiles differ between people with healthy aging, AUD, WKS, Alzheimer's disease, and other subtypes of dementias. AUD adds a burden to aging in the executive domain. Although AUD, WKS, and Alzheimer's disease all affect memory processes, the effects of Alzheimer's disease on mnemonic functions are greater than those observed in AUD and WKS.

Postmortem neuropathology

Normal aging decreases the brain's viability and increases its vulnerability to damage,[128,129] but neuronal loss is not a salient feature.[130-132] Instead, careful stereological studies have concluded that age-related changes in the central nervous system (CNS) in the cognitively intact, aging brain include alterations to neuron extensions (e.g., retraction of dendritic arbors and synapses);[133,134] deterioration of non-neuronal cells (e.g., oligodendrocytes, astrocytes, microglia);[135-138] and biochemical and molecular changes (e.g., reduced efficacy of neurotransmitters).[139-142] These effects of aging in the healthy brain differ from those seen with pathological aging due to neurological conditions such as Alzheimer's disease.[143,144] A cardinal pathological feature of Alzheimer's CNS tissue, which has been known for more than 100 years, is the progressive accumulation of insoluble fibrous materials, including extracellular plaques of beta-amyloid (A-beta), which has two major isoforms (A-beta-42 and A-beta-40), and intraneuronal neurofibrillary tangles composed of the microtubule-binding protein tau.[145-147] The cause, effect, and reciprocity of A-beta and tau accumulation with neurodegeneration and symptoms of dementia are the subject of ongoing debates.[49,57,59,148] Nevertheless, substantiation of an Alzheimer's diagnosis continues to require postmortem identification of these characteristic protein inclusions in regions including the entorhinal cortex and hippocampus, where they contribute to severe neuronal loss and salient impairment in memory consolidation of newly experienced events.[149,150] Neuropathological observations further suggest that neuronal loss in a specific area of the hippocampus (i.e., subfield CA1) may be a specific marker for Alzheimer's disease.[151-153]

Other proteinopathies also present with neuropathological inclusions. Lewy body dementia is characterized by presence of protein aggregates (Lewy bodies) containing alpha-synuclein,[154] whereas frontotemporal dementia is associated with tau and TDP-43 (transactive DNA binding protein of about 43 kDa) pathology in at least 50% of cases.[155-157] In vascular dementia, gross examination of the brain may reveal overt lesions, microinfarcts, or damage to blood vessels, and microscopic evaluation may detect accumulation of lipids or blood clots.[158,159] Other postmortem signs of vascular disease include white matter atrophy and calcification of arteries.[43,160,161]

A coordinated cross-sectional analysis of six community-based autopsy cohorts in the United States and the United Kingdom highlighted the complexity of the brain pathologies that underlie dementia. The analysis assessed 12 dementia-related pathologies in brains of those age 80 and older, including vascular pathologies (arteriolosclerosis, atherosclerosis, microinfarcts, lacunes, and cerebral amyloid angiopathy); Alzheimer's disease-related pathologies (Braak neurofibrillary tangle stage, Consortium to Establish a Registry for Alzheimer's Disease [CERAD] diffuse plaque score, CERAD neuritic plaque score, and hippocampal sclerosis); Lewy body dementia pathology; and TDP-43 pathology. Of the overall sample, which generally included more women than men, 40% had vascular-related pathology, 70% had Alzheimer's disease-related pathology, and 68% of the cohort had pathology co-occurrence.[162] Smaller studies similarly reported a high frequency of coincident neuropathology.[163,164]

WKS does not have clear neuropathological markers. Careful stereological approaches, however, have demonstrated neuronal loss in medial thalamus, mammillary bodies, pons,

medulla, and anterior-superior vermis of the cerebellum.[165,166] A series of neuropathological analyses compared the effects of alcohol per se to distinct neurological conditions associated with chronic alcohol consumption, including WKS, hepatocerebral degeneration, Marchiafava Bignami disease, and central pontine myelinolysis. The studies concluded that alcohol as such does not contribute to a progressive or irreversible pathology.[118,167-170] Instead, quantitative histological analyses of individuals with uncomplicated AUD often use the term "alcohol-related brain damage" to refer to the plastic CNS changes associated with chronic alcohol use as discrete from neurodegenerative disease.[171,172] Tissue loss occurring mainly in the frontal lobes and cerebellum of the brain in people with AUD is not associated with neuronal death.[173-177] Indeed, no changes in neuron numbers have been documented in brain tissue (e.g., hippocampus, basal ganglia, serotonergic raphe nuclei, cholinergic basal forebrain) from people with AUD without liver pathology, nutritional deficiencies, or other complications.[177-182] AUD-related neuropathological changes are instead largely accounted for by retraction of dendritic arbors and shrinkage of white matter.[173,174,183-188]

Alzheimer's disease-related protein markers (i.e., A-beta, tau) are not affected by alcohol consumption. For example, A-beta plaques were not increased in the brains of people who drank heavily (more than 6 drinks per day for at least 10 years).[189,190] Further, men who drank moderately (not more than 4 drinks/day or 14 drinks/week) showed less neurofibrillary tangle pathology compared with men who drank never or heavily.[191] In a study of individuals with thiamine deficiency who who drank alcohol chronically, neurofibrillary pathology was found in the nucleus basalis (which is affected in WKS) but not in any other brain region.[192] Further, heavy alcohol consumption (i.e., daily, socially disabling alcohol use, and continued drinking despite indisputable health-related or social damage) showed no statistically significant influence on the extent of alpha-synuclein pathology or incidence of total infarcts;[193] however, very heavy alcohol consumption (more than 32 drinks/week) may increase hemorrhagic stroke.[194]

In summary, evidence from postmortem histological analyses indicates that healthy CNS aging and AUD are not associated with significant neuronal loss, whereas Alzheimer's disease and WKS show regionally specific neurodegeneration. Based on postmortem evaluations, uncomplicated AUD does not contribute to archetypal Alzheimer's disease pathology characterized by the presence of protein inclusions.

Neuroimaging biomarkers

An advantage of in vivo neuroimaging over postmortem study is the ability to track individuals longitudinally, which permits evaluation of causative factors in CNS volume changes and the consequences of behavioral modifications (e.g., cessation of alcohol drinking). Cross-sectional and longitudinal magnetic resonance imaging (MRI) studies in adults have provided consistent evidence for systematic, age-related volume increases in spaces filled with cerebrospinal fluid (CSF)—i.e., sulci, fissures, and ventricles—that occur at the expense of gray matter and may accelerate with older age.[195-201] Brain gray matter structures exhibit differential patterns of aging, with convergent longitudinal data indicating an excessive vulnerability of prefrontal cortex.[202-206] Age-related volume deficits in thalamus and cerebellum occur at a slower rate than declines in cortical gray matter.[207-211] Gross white matter volume remains relatively stable across adulthood;[201,212-214] however, appropriate imaging modalities (e.g., fluid-attenuated inversion recovery, diffusion tensor imaging) demonstrate more hyperintense inclusions (i.e., white matter hyperintensities [WMH]),[215-218] and microstructural compromise in older relative to younger individuals.[219-222]

Cross-sectional neuroimaging reports support AUD-related volume shrinkage in specific brain structures, including frontal, temporal, and parietal cortices; diencephalon; brain stem; and cerebellum.[223-229] In contrast to results of postmortem analyses of neuronal numbers, neuroimaging studies describe significant volume deficits in people with AUD, relative to healthy controls, in hippocampus and basal ganglia (i.e., caudate, putamen, nucleus accumbens) that may be accounted for by white matter compromise.[224,230-235] Longitudinal studies that compare individuals with older age at AUD onset and relatively less lifetime alcohol use with individuals with younger age at AUD onset further support accelerated brain aging in frontal cortical volumes due to age–alcohol interactions and not just attributable to more years of alcohol misuse.[227,236-239] Other longitudinal studies show that alcohol abstinence is associated with brain integrity improvement (i.e., volume recovery), whereas relapse precipitates further volume shrinkage.[240-244] Individuals with AUD who relapse show continuing volume decline compared with those who achieve abstinence,[225,241,245,246] but even reduced drinking without achieving or maintaining complete abstinence can improve brain structure and function.[247] Similarly, a controlled longitudinal study that assessed individuals with AUD soon after withdrawal and then again after 2 weeks of sobriety suggested resolution of volume deficits specifically in hippocampal subfield CA2+3[248] (also see Zahr et al., 2019[232]; Lee et al., 2016[249]). This reversibility of volume deficits with abstinence is in stark contrast to the irrevocable progression of Alzheimer's disease.[250,251]

Acute Wernicke's encephalopathy also has characteristic changes evident on transverse relaxation time (T2)-weighted images showing bilateral, high signal intensities in the periaqueductal gray, mammillary bodies, thalamus, and hypothalamus.[252-254] Quantitative MRI documents a graded pattern of accruing volume deficits in hippocampus, thalamus, mammillary bodies, cerebellum, and pons as disease severity progresses from AUD to WKS.[230,255,256] Mammillary body shrinkage has been suggested as being able to differentiate WKS from Alzheimer's disease,[257,258] as have diffusion tensor imaging

(DTI) metrics indicating abnormalities in anterior thalamus to hippocampus white matter tracts.[259]

Deviations of hippocampal volume from normal age-related decline have been identified as a sensitive indicator of Alzheimer's disease pathology.[22,234,260,261] Indeed, atrophy of entorhinal cortex and hippocampus may distinguish Alzheimer's disease from healthy aging with up to 90% accuracy;[262,263] further, the rate and extent of CA1 atrophy may help distinguish Alzheimer's disease from other forms of dementia.[241,264-267] Longitudinal studies suggest that the pattern of gray matter atrophy in people with MCI who are later diagnosed with Alzheimer's disease mimics the pattern of atrophy observed in Alzheimer's disease but is less extreme. However, in people with MCI who do not eventually receive an Alzheimer's disease diagnosis, the pattern of gray matter atrophy is more comparable to that observed in healthy elderly individuals without dementia.[268-270] Similarly, detrimental changes in regional (e.g., fornix, uncinate, cingulum) diffusivity in MCI quantified using DTI are less pronounced than those observed in people with Alzheimer's disease.[114,271,272]

A research framework for diagnosing Alzheimer's disease, released by the National Institute on Aging in 2018, integrated neuroimaging biomarkers A, T, and N. In this framework, A represents A-beta plaques determined by cortical amyloid PET ligand binding (or CSF A-beta-42 levels); T represents fibrillar tau protein, determined by cortical tau PET ligand binding (or CSF phosphorylated tau [p-tau] levels); and N represents neuronal injury or neurodegeneration determined with fluorodeoxyglucose PET hypometabolism or MRI volume (typically hippocampal) atrophy.[273-275] These three markers are used to distinguish among eight dementia profiles: normal, healthy individuals (A-T-N-); people with a condition along the Alzheimer's disease continuum (A+T-N-; A+T-N+; A+T+N-; A+T+N+); and people with non-Alzheimer's changes (A-T+N-; A-T+N+; A-T-N+).[57,276]

Vascular dementias (which include at least six subtypes) are identified on MRI by presence of infarcts, small cavities (lacunes), and WMH.[277-280] WMH are considered a neuroimaging feature of cerebral small vessel disease that can increase the risk for stroke and vascular dementia.[281,282] As they are ubiquitous and heterogeneous, however, a better characterization of the extent, distribution, and cognitive correlates of WMH is necessary.[283-285] In support of a high co-occurrence of Alzheimer's disease and vascular dementias, a literature review found a strong relationship between presence of amyloid and WMH burden[286] (also see Eloyan et al., 2023[287]).

Although separate structural neuroimaging studies in people with AUD, WKS, or Alzheimer's disease report gray matter volume loss in common regions, including hippocampus,[258,288,289] a direct comparison among these patient groups demonstrates that hippocampal volume loss in people with AUD relative to Alzheimer's disease is less severe.[290] Further, PET markers that characterize Alzheimer's disease are not elevated in people with AUD. Two PET studies using the Pittsburgh Compound-B ([^{11}C]PiB) ligand found no significant differences in global A-beta loads between people with AUD and healthy control study participants[291,292] (also see Mendes et al., 2018[293]). Another report found that compared with no drinking, moderate drinking (1 to 13 drinks/week) was associated with lower [^{11}C]PiB-determined A-beta deposition.[294] In contrast, people with AUD had larger WMH volumes than did healthy controls, suggesting an increased cerebrovascular risk in AUD.[207,292]

In summary, healthy aging is characterized by nonlinear gray matter volume decreases, particularly in frontal regions; slower white matter decline; and a greater incidence, compared to younger brains, of WMH.[227,295-298] AUD can amplify the severity and extent of age-related volume decline, especially in frontal regions, but abstinence is associated with significant volume recovery.[246,299] In vivo diagnosis of Alzheimer's disease necessitates PET imaging, but available evidence does not support AUD as contributing to Alzheimer's disease PET markers. In vivo MRI of individuals with Alzheimer's disease can demonstrate greater than age-corrected hippocampal atrophy, but deviations from age-related changes can be challenging to quantify. Instead, emerging data suggest that hippocampal subfield analyses (e.g., effects on CA1 in Alzheimer's disease and on CA2+3 in AUD) may help with future differential diagnoses.

CSF and blood biomarkers

The National Institute on Aging research framework supports CSF quantification of extracellular A-beta-42 and p-tau for accurate and early diagnosis of Alzheimer's disease, but optimization and standardization of these measures is in progress.[300-302] CSF A-beta-42 levels are low in people with Alzheimer's disease compared to unaffected controls and reflect an increase in CNS amyloid plaques.[303-305] Low CSF levels of A-beta-42 also can predict MCI and conversion from MCI to Alzheimer's disease.[306,307] As levels of CSF A-beta-42 are also low in Lewy body, vascular, and frontotemporal dementias, however, A-beta isoforms are being explored to help to differentiate dementia subtypes.[308-310] Levels of CSF tau, p-tau, and their epitopes are high in people with Alzheimer's disease compared to unaffected subjects and may indicate hippocampal atrophy, but levels of these CSF proteins are also high relative to healthy controls in other neurodegenerative diseases.[311-313] Combinations and ratios (e.g., A-beta-42/A-beta-40) of CSF A-beta-42, total tau, and p-tau and their variants are under investigation to improve success of differential diagnoses.[314,315]

Total tau is significantly elevated in people with acute Wernicke's encephalopathy, but the overall pattern of CSF changes (involving A-beta, total tau, and p-tau) can clearly distinguish acute and chronic WKS from Alzheimer's disease.[316] CSF tau and A-beta markers are present in only 11% of AUD patients with cognitive deficits;[317] conversely, alcohol misuse is rarely observed in those with Alzheimer's disease

biomarkers.[318] Thus, CSF tau and A-beta markers may be useful in differentiating alcohol-related cognitive disorders from Alzheimer's disease.[319]

Although neuroimaging and CSF markers approved by the U.S. Food and Drug Administration can aid in detection and diagnosis of Alzheimer's disease, the clinical implementation of these testing modalities is limited because of their availability, cost, and perceived invasiveness.[320] Blood-based markers are also in development for earlier, faster, and more accessible diagnoses.[321] Associations between blood and CSF tau and A-beta and other disease markers, however, and their ability to help with differential diagnoses are not fully established.[322-325]

Summary of human studies

The consensus among studies from multiple disciplines is that AUD can increase the risk for dementia, but not necessarily the risk of Alzheimer's disease. A review of clinical and epidemiological data suggests that criteria and nomenclature of dementia subtypes need improvement. Neuropsychological and biological markers that can differentiate dementia subtypes are in progress but currently limited. Whether alcohol misuse contributes to an added burden on pre-existing Alzheimer's disease remains an open and ongoing research question, which may be approached in animal models. Indeed, basic science strategies that can control alcohol exposure may help clarify controversies, including whether alcohol in the context of genetically induced Alzheimer's disease pathology changes the extent, distribution, or signaling pathways of relevant biomarkers.

Translational Studies

Rodent models of AUD

In contrast to the human brain, the rat brain increases in weight and length with advancing age and demonstrates continued growth in older (e.g., age 763 days) relative to younger (e.g., age 109 days) rodents.[326,327] Longitudinal imaging studies that followed animals for up to 19 months confirm accrual of body weight and total brain volume with increasing age in wild-type Wistar rats, alcohol-preferring (P) and non-preferring (NP) strains derived from Wistar rats, and Fischer 344 (F344) rats.[220,228,328-330] MRI studies further show an aging-related pattern in rats contrary to that observed in humans: Total CSF, gray matter, and white matter volumes continue to increase with older age.[228,331] These fundamental differences in CNS aging between rodents and humans are critical to model in studies that consider the combined effects of ethanol (EtOH) exposure and Alzheimer's disease-related pathology.

Several susceptible brain regions have been demonstrated in rodents exposed to high EtOH levels via intragastric,[332] intraperitoneal (i.p.),[333,334] or vapor[335,336] protocols. Immunohistochemical staining procedures highlight degenerative effects of EtOH on corticolimbic circuitry.[337-342] By contrast, unbiased screening approaches that indicate neuronal activity (e.g., glucose utilization, c-Fos expression) but not loss identify different regions affected by EtOH, including thalamus, colliculi, cerebellum, and pons.[343-346] Longitudinal neuroimaging findings consistently report ventricular enlargement in response to binge and chronic EtOH exposure that is reversible upon abstinence.[228,330,347-349] Indeed, among regions demonstrating reduced volume following EtOH exposure (e.g., retrosplenial and cingulate cortices, dorsal hippocampi, central and ventroposterior thalami, corpus callosum), most show significant recovery with abstinence.[350,351] Volumes of the colliculi and periaqueductal gray, however, show persistent volume deficits with abstinence.[350,351] Although the colliculi may be relevant to human AUD, they have rarely been investigated in humans, possibly because of the challenges in visualizing and quantifying colliculi by MRI.[352]

Relatively few papers have explored the effects of EtOH on the aged rodent brain. Following a single i.p. EtOH dose, older (18 months) compared with younger (postnatal days 70 to 72) Sprague Dawley rats showed greater EtOH-induced ataxia (accelerating rotarod, aerial righting reflex) and cognitive impairment (i.e., longer latency to locate submerged platform on the Morris water maze).[353] However, against expectations, a longitudinal in vivo study of F344 rats exposed to intragastric EtOH for 4 days[354] showed greater transient tissue volume compromise in young rats (age 4 months) compared to older rats (age 17 months).[331] By contrast, EtOH administration alters markers of astrocytes and microglia more significantly in older than younger animals. For example, chronic moderate EtOH exposure (daily 2 g/kg, i.p. doses for 45 days) increased glial fibrillary acidic protein (GFAP, an astrocyte protein expression marker) to a greater extent in older (age 19 months) than younger (age 3 months) Wistar rats.[355] Similarly, a microglial mRNA marker that increased in response to EtOH resolved with abstinence in young but not older C57BL/6J mice[356] (also see Marsland et al., 2022[357]).

Rodent models of Alzheimer's disease

Several genetically modified (i.e., transgenic) mouse models of Alzheimer's disease are now available. The first models used various constructs to overexpress amyloid precursor protein (APP), which is processed in the body by enzymes (i.e., beta- and gamma-secretases) to generate soluble amyloid peptide (A-beta) fragments.[358] Mice with overproduction of total A-beta from APP exhibit extracellular A-beta deposits reminiscent of plaques in human brains as well as cognitive dysfunction.[359-361] However, these animals did not have neurofibrillary tangles or show neuronal loss. Second-generation mutant mice included overexpression of presenilin (PS), a constituent of the gamma-secretase complex that cleaves APP.[362] PS1 overexpression alone did not induce A-beta pathology;[363] however, the combined expression of APP and PS1 increased pathogenic A-beta production and deposition, behavioral deficits, and neuronal

loss.[364-367] One of these models was the 5XFAD mouse line, which expresses five human APP and PS1 transgenes and results in mice with A-beta pathology, gliosis, synaptic degeneration, neuronal loss, and progressive cognitive deficits as early as 4 months of age.[368] Despite their aggressive phenotypes, these models also failed to develop neurofibrillary tangles. In efforts to replicate neurofibrillary tangle pathology, a mouse line was created that carried targeted insertions (knock-in mutations) of PS1, APP, and microtubule-associated protein tau (i.e., 3xTg-AD mice).[369] The 3xTg-AD mouse line is a well-validated animal model that develops rapid, age-dependent, and progressive Alzheimer's-like neuropathology, including A-beta and tau tangles.[370-372]

Although widely used, these models imitate only certain aspects of human Alzheimer's disease pathology.[373-375] Further, the amyloid peptides generated by mice are distinct from those produced by the human brain.[376] Such gaps have led to a program initiated by the National Institute on Aging—the Model Organism Development and Evaluation for Late-Onset Alzheimer's Disease (https://www.model-ad.org)—to fund development of Alzheimer's disease mouse models that better recapitulate the human disease.

Rodent models of AUD and Alzheimer's disease

Only a few studies have evaluated how EtOH may exacerbate Alzheimer's-related behavior and brain pathology in wild-type rodents. Compared to unexposed mice, wild-type C57BL/6J mice exposed to EtOH (1 month, free access to water, 10% or 20% EtOH) showed impaired spatial memory and elevated hippocampal p-tau, but no change in total tau.[377] Similarly, wild-type, male C57BL/6J mice exposed to both EtOH (via liquid diet for 7 weeks at 28% of total calories) and thiamine deficiency demonstrated nonspecific, whole-brain increases in A-beta (both A-beta-42 and A-beta-40 isoforms) protein levels compared to unexposed mice[378] (also see Zhao et al., 2011[379]). Finally, compared with unexposed animals, Sprague Dawley rats exposed to EtOH (via liquid diet for 5 weeks at about 36% of total calories) showed increased expression of APP and beta-site APP-cleaving enzyme 1 (BACE1, which is critical for A-beta expression) in hippocampus, cerebellum, and striatum.[380] Of note, nonspecific, elevated levels of A-beta also have been observed in response to other age-related pathologies (e.g., hypertension, diabetes[381,382]), and elevations in p-tau can occur in response to other, particularly anesthetic, psychoactive agents.[383,384]

Findings observed in wild-type animals appear to be exaggerated in transgenic mice. For example, APP/PS1 mice exposed to EtOH (drinking in the dark for 1 month), compared to vehicle-treated APP/PS1 animals, showed greater memory deficits (i.e., Morris water maze performance), higher whole-brain APP and BACE1 levels, and enhanced plaque formation.[385] Similarly, compared with unexposed mice, APP/PS mice exposed to 10 weeks of moderate EtOH in a two-bottle choice design showed deficits in nest building (but not in an object location memory task), and a higher frequency of A-beta deposition and plaques in hippocampus.[386] Also in APP/PS transgenic mice, binge EtOH treatment during adolescence (via four i.p. injections per week of 2.5 g/kg EtOH during postnatal days 20 to 60) increased A-beta RNA and protein expression in the hippocampus at ages 6 and 12 months.[387] In 3xTg-AD mice—the only transgenic model able to produce both A-beta and tau markers—EtOH exposed (via 4-month, free access to water or 25% EtOH), compared with saccharin-exposed (control) 3xTg-AD mice, showed impaired spatial memory on the Morris water maze and upregulated A-beta-42/40, total tau, and p-tau 1 month after EtOH exposure.[388] Another study showed that EtOH exposure (6 weeks of 4 days/week vaporized EtOH) to 3xTg-AD mice hastened cognitive impairment and increased levels of a different protein marker, alpha-synuclein (relevant to Lewy body dementias).[389]

Recent translational work highlights sex differences in the interaction of EtOH with Alzheimer's disease-related pathology. EtOH exposure caused greater cognitive impairment in female than male "middle aged" (ages 6 to 9 months) wild-type C57BL/6J mice,[390] which was associated with an increase in hippocampal amyloid levels.[391] In mice with abnormal tau deposition (i.e., PS19 model with the T34 tau isoform), 16 weeks of intermittent access to water containing 20% EtOH increased the excitability of the locus coeruleus more in female than male mice.[392] Finally, 3xTg-AD adolescent and adult mice exposed to EtOH showed EtOH-related increases in total and hyperphosphorylated tau in female mice but not in male mice, which were hypothesized to be related to impaired lysosome function.[393,394] These recent papers demonstrating EtOH effects in only female transgenic mice[393,394] acknowledged previous findings that total tau and p-tau were increased in both sexes of 3xTg-AD mice,[388] but did not comment on the underlying reasons for such discrepancies. Indeed, the relevance of sex-related findings in transgenic rodents to the human condition await a better understanding of the pathological mechanisms underpinning Alzheimer's disease.

Conclusions

Limitations of the current narrative review are that it failed to address all nuances of the potential relationship between alcohol misuse and dementia risk. For example, the contributions of a genetic predisposition to Alzheimer's disease (i.e., presence of the apolipoprotein E epsilon4 allele, the major genetic risk factor) to the various metrics were not considered.[92,395] Further, an emerging literature showing a relationship between liver pathology—including alcohol-related liver disease—and Alzheimer's disease was not explored.[396-398]

This literature review indicates that chronic alcohol misuse accelerates brain aging and contributes to cognitive impairments, including those in the mnemonic domain also

affected in Alzheimer's disease. The current literature analysis, however, agrees with a 2001 review published in this journal that alcohol misuse does not increase the risk for Alzheimer's disease per se.[399] Whether alcohol misuse or AUD increase the risk for alcohol-related or other forms of dementia may be clarified by improvements in neuropsychological tests or biomarkers better able to differentiate dementias in vivo.

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Sleep-Related Predictors of Risk for Alcohol Use and Related Problems in Adolescents and Young Adults

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PURPOSE: Growing evidence supports sleep and circadian rhythms as influencing alcohol use and the course of alcohol use disorder (AUD). Studying sleep/circadian–alcohol associations during adolescence and young adulthood may be valuable for identifying sleep/circadian-related approaches to preventing and/or treating AUD. This paper reviews current evidence for prospective associations between sleep/circadian factors and alcohol involvement during adolescence and young adulthood with an emphasis on the effects of sleep/circadian factors on alcohol use.
SEARCH METHODS: The authors conducted a literature search in PsycInfo, PubMed, and Web of Science using the search terms "sleep" and "alcohol" paired with "adolescent" or "adolescence" or "young adult" or "emerging adult," focusing on the title/abstract fields, and restricting to English-language articles. Next, the search was narrowed to articles with a prospective/longitudinal or experimental design, a sleep-related measure as a predictor, an alcohol-related measure as an outcome, and confirming a primarily adolescent and/or young adult sample. This step was completed by a joint review of candidate article abstracts by two of the authors.
SEARCH RESULTS: The initial search resulted in 720 articles. After review of the abstracts, the list was narrowed to 27 articles reporting on observational longitudinal studies and three articles reporting on intervention trials. Noted for potential inclusion were 35 additional articles that reported on studies with alcohol-related predictors and sleep-related outcomes, and/or reported on candidate moderators or mediators of sleep–alcohol associations. Additional articles were identified via review of relevant article reference lists and prior exposure based on the authors' previous work in this area.
DISCUSSION AND CONCLUSIONS: Overall, the review supports a range of sleep/circadian characteristics during adolescence and young adulthood predicting the development of alcohol use and/or alcohol-related problems. Although sleep treatment studies in adolescents and young adults engaging in regular and/or heavy drinking show that sleep can be improved in those individuals, as well as potentially reducing alcohol craving and alcohol-related consequences, no studies in any age group have yet demonstrated that improving sleep reduces drinking behavior. Notable limitations include relatively few longitudinal studies and only two experimental studies, insufficient consideration of different assessment timescales (e.g., day-to-day vs. years), insufficient consideration of the multidimensional nature of sleep, a paucity of objective measures of sleep and circadian rhythms, and insufficient consideration of how demographic variables may influence sleep/circadian–alcohol associations. Examining such moderators, particularly those related to minoritized identities, as well as further investigation of putative mechanistic pathways linking sleep/circadian characteristics to alcohol outcomes, are important next steps.

KEYWORDS: alcohol; adolescent; sleep; circadian rhythm; young adult; experimental model; longitudinal studies; research design

Abundant cross-sectional data indicate that alcohol use and related problems are accompanied by disruptions to sleep and circadian rhythms.[1] Alcohol's negative impacts on sleep are well established, especially in adults, and a smaller body of literature also reports alcohol's disruption of circadian rhythms.[2-4] Growing evidence supports sleep and circadian factors as influencing alcohol use and related problems, including as risk factors for the initial development of use and problems, as predictors for relapse in individuals with alcohol use disorder (AUD), and as targets for intervention.[2,5-7] Given the marked changes in sleep and circadian rhythms that occur throughout adolescence into young adulthood,[5] paralleling the time frame when initial alcohol use and development of alcohol-related problems are most likely to occur,[8] there may be particular value to studying the association between sleep/circadian rhythms and alcohol during this developmental stage.

Sleep and Circadian Changes in Adolescence and Beyond

As a result of living on a rotating planet with alternating light and dark periods, humans and most other living organisms have evolved to experience internal biological rhythms lasting approximately 24 hours.[9] These circadian rhythms modulate the timing of many, if not most, physiological, behavioral, and psychological processes, including the sleep-wake cycle, with the goal of optimizing temporal relationships with the environment and with one another. Notably, the timing of circadian rhythms is not static but shows developmental changes. Starting with the onset of puberty, the timing of sleep and circadian rhythms shifts later throughout adolescence, peaking around age 20 before reversing course and slowly shifting earlier over the rest of the life span.[10,11] The changes in sleep timing are driven by both biological and sociocultural factors and thus can vary based cross-nationally[12] and on sociodemographic characteristics.[11,13] Biological factors include the changes in circadian rhythms as well as changes in homeostatic sleep propensity, which accumulates more slowly during adolescence.[14] Exposure to blue light (e.g., via electronic devices) in the evening can exacerbate these tendencies toward later sleep and circadian timing.[15,16]

Although the need for sleep remains relatively stable during this period—with recommendations for 8 to 10 hours/night in youth ages 13 to 17 and for 7 to 9 hours/night in people age 18 and older—actual sleep duration tends to decrease, especially on school/work nights.[14,17] This reduction in sleep duration is driven in part by a mismatch between the tendency for later sleep/circadian timing and relatively early school schedules, particularly during middle school and high school. This mismatch, termed circadian misalignment or social jet lag, not only results in insufficient sleep duration, but also can contribute to difficulty falling asleep on school nights, daytime sleepiness on school days, and large swings in sleep timing and duration on weekdays versus weekends.[14] Such swings tend to manifest as later sleep timing and shorter sleep duration, especially for those with later circadian timing.[18] Although the effects of early school start times are most systematic during secondary education, circadian misalignment and the associated constellation of sleep problems can persist well after high school. Regardless of etiology, insomnia, insufficient sleep, and social jet lag remain prevalent in the years after high school graduation into people's twenties,[18-20] although prevalence varies based on sociodemographic characteristics.[21]

Sleep is multifactorial, and as illustrated above, different facets of sleep are interrelated in complex ways.[22,23] Circadian misalignment and social jet lag are often accompanied by a constellation of sleep-related problems and thus cannot be adequately captured by only assessing sleep quality, sleep duration, or sleep timing, especially if not distinguishing between school days or workdays and free days.

Alcohol Trajectories in Adolescence and Beyond

The developmental span from adolescence to young adulthood is a time of increasing alcohol use and related problems.[8] Alcohol use then tends to decline in early adulthood as individuals begin to "mature out" due to increases in adult responsibilities.[24] Further, both earlier initiation of alcohol use and more rapid progression from initiation to intoxication have been found to predict problematic alcohol use later on.[25-27] Multiple explanatory mechanisms are thought to underlie the onset and progression of risky alcohol use in adolescence through early adulthood. In particular, heightened sensation seeking and impulsivity have been consistently identified as potential risk factors for problematic alcohol use[28-33] and are related to sleep and circadian factors.[34,35]

Overview of Alcohol's Effects on Sleep

The effects of alcohol on sleep and, to a lesser extent, circadian rhythms in adult samples have been thoroughly and recently reviewed,[2-4] so are only briefly discussed here. Given the bidirectional relationships between sleep and alcohol use, a brief summary of the evidence for alcohol's effects on sleep and circadian rhythms is warranted as it provides important context in interpreting observational data where it is impossible to fully parse these bidirectional effects.

Alcohol administration studies in adults have assessed alcohol's acute effects on sleep via polysomnography, which measures brain activity (electroencephalography [EEG]), eye movements, muscle activity, and cardiac activity. These studies found that during the first half of the night, alcohol tends to shorten the time it takes to fall asleep (sleep onset latency [SOL]), reduce nighttime wakefulness (i.e., decrease wake after sleep onset [WASO]), decrease rapid eye movement (REM) sleep, and increase the deepest of the non-REM sleep stages (i.e., slow-wave sleep).[2] (See **Box: Glossary of Sleep-related Terms** for more detailed definitions.) However, during the second half of the night, alcohol tends to acutely increase WASO and reduce sleep efficiency (the percentage of time spent asleep relative to the time spent attempting to sleep), while leading to a rebound in REM sleep.[2] Overall, polysomnography studies suggest that adults spend more time awake on nights after consuming alcohol.[2] Some sex differences in the acute effects of alcohol have been noted, as described below.

Acute alcohol effects in adolescents have been much less studied, but findings suggest some distinctions from the effects observed in adults. In a study with 24 participants ages 18 to 21 (12 women) with a mean breath alcohol content of 0.084% at lights out, alcohol's effects were broadly similar but with less evidence of benefits for sleep. Specifically, adolescents did not exhibit the decrease in SOL or the REM rebound,[36] and although alcohol appeared to increase delta power (EEG activity in the 1–4 Hz range; typically highest during slow-wave sleep) during the first few sleep cycles, it simultaneously increased alpha power (EEG activity in the 8–13 Hz range; associated with quiet wakefulness) in frontal regions.[37] This alpha-delta pattern in response to alcohol has been observed in some but not all prior studies[38,39] and is thought to reflect disrupted sleep. No sex differences were reported.

As reviewed by Koob and Colrain,[2] alcohol's effects on sleep—when alcohol use is more chronic and/or when people who chronically use alcohol (i.e., patients with AUD) abstain from drinking—can diverge from the acute effects of alcohol in complex ways too nuanced to adequately review here. Generally, chronic alcohol use is associated with worse sleep (e.g., more insomnia, longer SOL and WASO), although sleep may intermittently improve on drinking nights; similarly, abstinence is typically associated with initial worsening of sleep with some incremental improvement over time.[2] Various sleep abnormalities persist in individuals with AUD, even with long-term abstinence (> 30 days). A recent meta-analysis of cohort studies in broader samples underscores the general conclusion that chronic alcohol use does not improve sleep overall, and likely increases the likelihood of developing sleep disorders over time.[40]

Although intensive longitudinal studies cannot confirm causality or directionality, analyses of day-to-day alcohol–sleep associations in young adults suggested that drinking on a given day was associated with later sleep timing that night.[41,42] Interestingly, such analyses offered mixed evidence for whether drinking worsened[43] or improved[42] sleep. Additionally, some studies in young adults have shown that cannabis use may mitigate alcohol's

Glossary of Sleep-related Terms

Actigraphy: Noninvasive and objective method of measuring rest-activity patterns, and thereby estimating sleep-wake characteristics, via a wearable device containing an accelerometer. Most typically worn on the wrist.

Chronotype: Tendency toward relatively earlier or relatively later timing of the circadian clock, often as indexed by timing of the sleep-wake schedule. Conceptually overlaps with circadian preference and/or morningness-eveningness—the self-reported preference for relatively earlier (morningness) or later (eveningness) patterns of activity and sleep.

Circadian misalignment: Mismatch between the timing of the behavioral sleep-wake schedule and that of the circadian clock, most obviously observed in the context of shiftwork and jet lag.

Eveningness: Self-reported preference for relatively later timing of sleep and activity. In contrast to morningness, a self-reported preference for relatively earlier timing of sleep and activity. See chronotype.

Polysomnography: A multiparameter assessment of sleep that includes electroencephalography (EEG) to assess brain activity, electrooculography (EOG) to assess eye movements, electromyography (EMG) to assess muscle activity, and electrocardiography (ECG) to assess cardiac activity. Often respiratory airflow, respiratory effort, and pulse oximetry are also measured. Typically applied in laboratory-based settings, although streamlined polysomnography-type devices are increasingly used in home settings.

Sleep efficiency: The percentage of time spent asleep relative to the time spent attempting to sleep.

Sleep onset latency (SOL): The amount of time it takes to fall asleep.

Slow-wave sleep: The deepest of three stages of non-rapid eye movement (non-REM) sleep.

Social jet lag: A specific type of circadian misalignment in which school and/or work obligations cause a mismatch between the imposed sleep-wake schedule on school days or workdays, whereas individuals return to their desired sleep-wake schedules (relatively more aligned with their circadian clocks) on free days. More common are individuals with a late chronotype (tendency toward evening circadian preference).

Wake after sleep onset (WASO): The amount of time spent awake during nighttime awakenings that occur after initially falling asleep.

effects on sleep,[43,44] although these studies require replication, and the relevant mechanisms remain unknown.

Alcohol's effects on sleep also depend on the timing of alcohol consumption; for example, a study in middle-aged men administered alcohol 6 hours before bedtime found no benefit for SOL.[45] This likely was due to a combination of the temporal dynamics of the biphasic response to alcohol and circadian variation in the response to alcohol. While the literature on alcohol effects on circadian rhythms is more limited, particularly in humans,[3,4] studies have suggested disruption of melatonin and core body temperature rhythms. Multiple animal studies have indicated that acute and chronic alcohol use disrupted the circadian system's response to light, which is the most important cue (zeitgeber, or time giver) for entraining to the 24-hour day.[46,47]

Although parallel effects in humans were not supported by one study in healthy adults reporting light or regular but not heavy alcohol use,[48] more recent work suggested reduced retinal responsivity to light in a group of adults who drank heavily.[49] Light or regular drinking has previously been defined as "consumption of one to five standard alcoholic drinks/week" and no more than three episodes of binge drinking in the past year.[49] Heavy drinking has been defined by the National Institute on Alcohol Abuse and Alcoholism as five or more drinks on any day or 15 or more drinks per week for men, or four or more drinks on any day or eight or more drinks per week for women (see https://go.nih.gov/TiogZz9). However, there is no standardized definition of either "light/regular drinking" or "heavy drinking" across the studies described in this article.

The present paper reviews current evidence for prospective associations between sleep/circadian factors and alcohol involvement during adolescence and young adulthood, with an emphasis on the effects of sleep/circadian factors on alcohol use and related outcomes. This focus was selected in part because identifying modifiable sleep–alcohol relationships during this developmental period offers the potential for shifting the trajectory for alcohol-related problems before they develop into chronic AUD. This article also describes and discusses potential mechanisms by which sleep may influence alcohol use and problems, as well as potential important differences in sleep–alcohol associations based on key moderators, such as assigned sex at birth; lesbian, gay bisexual, transgender, queer/questioning, intersex, and asexual (LGBTQIA+) identities; and racial and ethnic identities.

Methods

Search Methods

The initial search of the existing literature was conducted on July 18, 2022, in PsycInfo, PubMed, and Web of Science using the search terms "sleep" and "alcohol" paired with "adolescent" or "adolescence" or "young adult" or "emerging adult," in the title or abstract fields; results were restricted to English-language articles but had no restriction by date. Next, the search was narrowed by including only articles that had a prospective/longitudinal or experimental design, included a sleep-related measure as a predictor, assessed an alcohol-related measure as an outcome, and had a sample primarily composed of adolescents and/or young adults. Based on these search terms, the resulting ages of participants in the articles ranged from ages 12 to 30. Table 1 offers information on age ranges in specific studies. Two of the authors completed this step by conducting a joint review of candidate article abstracts.

Results of the Literature Search

The initial search resulted in 720 articles (174 in PsycInfo, 305 in PubMed, and 241 in Web of Science). After review of the abstracts to identify articles that met all the key search criteria, the list was narrowed to 27 articles reporting on observational longitudinal studies and three articles reporting on experimental studies (specifically, two intervention trials). Noted for potential inclusion were 35 additional articles that reported on studies with alcohol-related predictors and sleep-related outcomes, and/or reported on candidate moderators or mediators of sleep–alcohol associations. An additional 104 articles cited here were identified via a variety of methods, including review of relevant article reference lists and prior exposure based on the authors' previous work in this area. Finally, while this review focused on sleep/circadian–alcohol associations in human studies, a few select findings from three animal studies[46,47,50] were included when they appeared particularly complementary to the human findings and/or helped speak to a gap in the human literature.

Results of the Reviewed Studies

Longitudinal Sleep and Alcohol Studies

Overall, the existing literature—based on 27 articles, including three intensive longitudinal studies—provides consistent evidence that a range of sleep/circadian factors during adolescence predicts later alcohol involvement. These included difficulties with falling or staying asleep, lower overall sleep quality, shorter sleep duration, daytime sleepiness, later sleep timing and/or chronotype (i.e., tendency for relatively earlier or later sleep-wake timing), and variable sleep timing and/or social jet lag (see **Box: Glossary of Sleep-related Terms**). Alcohol-related outcomes assessed included metrics of both quantity and frequency of use, binge or heavy drinking episodes, alcohol intoxication, alcohol-related consequences/problems, AUD symptoms, and alcohol craving. Table 1 provides a summary of the longitudinal studies, including sample composition, study design, and timescale; which multidimensional sleep variables were predictive of alcohol outcomes; and whether differences across assigned sex, gender identity, and racial/ethnic identity were assessed.

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Fucito et al.[41]	2018	42 college students with concerns about their sleep, ≥ 1 occasion of heavy drinking in the past month	*M* age = 20.5 (*SD* = 1.31); 48% female; 4% Asian, 5% Black, 5% more than one race, 2% other, 69% White; 19% Hispanic/Latino	Intensive longitudinal; 7day protocol	Days	-	Y	Y	Y	-	-	-	Shorter sleep duration (actigraphy), earlier wake time (actigraphy), and better sleep quality all predicted more drinks the following day. In full model including all sleep variables, only wake time, sleep quality, and alertness upon waking predicted later alcohol use.	Tested differences across gender identity in sleep and alcohol variables (none significant)
Graupensperger et al.[51]	2022	409 young adults reporting past-month simultaneous use of alcohol and cannabis use and drinking alcohol ≥ 3 times in past month	*M* age = 21.6 (range = 18–25); 51% female; 16% Asian, 4% Black, 11% more than one race, 4% other, 48% White; 16% Hispanic/Latino	Intensive longitudinal; five 14-day bursts of twice-daily surveys separated by 4 months (i.e., 70 days total across 16 months)	Days, months, years	-	-	-	-	-	Y	-	At burst-level, shorter sleep duration was associated with greater alcohol use. At all three levels (person, burst, and daily), shorter sleep duration was associated with stronger morning alcohol craving. At burst and daily levels, shorter sleep duration was associated with stronger afternoon alcohol craving.	None tested
Hasler et al.[170]	2014	696 participants from study at the Pittsburgh Adolescent Alcohol Research Center. At baseline, 347 participants with current AUD (AUD+); 349 participants with no current or past AUD (AUD-)	AUD+: *M* age = 16.7 (range = 12–19); 37% female; < 1% Asian, 12% Black, 87% White AUD-: *M* age = 15.8 (range = 12–19); 57% female; < 1% Asian, 25% Black, < 1% Native American, 75% White	Longitudinal; data from baseline, 1-, 3-, and 5-year follow-up assessments	Years	Y	Y	-	-	-	N	-	In AUD- group, greater insomnia at baseline predicted increase in AUD symptoms at 1-year follow-up, while greater variability in weekday-weekend sleep duration predicted increases in AUD symptoms at 3- and 5-year follow-up.	AUD group differences reported across racial groups
Hasler et al.[54]	2016	707 children in Center for Education and Drug Abuse Research (CEDAR) study	*M* age = 11.4 (range = 9–13); 28.9% female; 21% Black, 79% White	Longitudinal; data from eight assessments spaced 2–3 years apart. Last assessment at approximately age 30	Years	Y	Y	-	-	-	-	-	More restless sleep predicted earlier onset of alcohol use. More variable sleep timing predicted earlier onset of AUD.	Sleep differences reported across racial groups; no significant differences across sex

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Hasler et al.[53]	2017	729 adolescents in National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) study	*M* age = 15.9 (range = 12–21); 51% female; 7% Asian, 12% Black, 41% other, 75% White; 12% Hispanic/Latino	Accelerated longitudinal design; data from baseline and 1–year follow-up assessments	Months	N	N	N	Y	-	Y	-	Greater eveningness, later bedtime (weekday and weekend), and shorter weekday sleep duration all predicted higher severity of binge alcohol use.	Sleep differences reported across racial and ethnic groups
Hasler et al.[52]	2017	93 male participants from Pitt Mother & Child Project originally recruited in infancy	Assessments at age 20 and age 22; race/ethnicity not reported	Longitudinal; data from two time points (age 20 and age 22)	Years	-	-	-	Y	-	-	-	An indirect path was observed from circadian preference at age 20 to alcohol use and dependence at age 22 via the neural (medial prefrontal cortex) response on a monetary reward task. However, circadian preference at age 20 did not directly predict alcohol use or dependence at age 22.	None tested
Hasler et al.[5]	2022	831 adolescents in NCANDA study	*M* age = 16.2 (range = 12–21); 50.9% female; 7% Asian American, 12% Black, 41% other, 75% White; 12% Hispanic/Latino	Accelerated longitudinal design; data from baseline through 5-year follow-up (six annual assessments)	Years	N	N	Y	Y	-	Y	-	Greater eveningness, more daytime sleepiness, later weekend sleep timing, and shorter sleep duration (weekday/weekend) all predicted more severe alcohol binge drinking the following year. Sleep associations with binge severity differed between middle/high school versus post-high school adolescents.	Sleep differences reported based on sex; no differences across racial groups were assessed
Haynie et al.[67]	2018	2,785 high school students from the NEXT Generation Health Study	Age not reported; 55% female; 14% Black, 5% Other, 62% White; 19% Hispanic/Latino	Longitudinal; data from 10th, 11th, and 12th grades (Waves 1–3)	Years	Y	-	-	Y	-	N	-	Later chronotype and greater social jet lag both predicted greater likelihood of alcohol use and heavy episodic drinking (Wave 2 to Wave 3 only). Sleep duration did not predict subsequent alcohol involvement.	Sleep differences reported across gender identity and racial and ethnic groups in Supplement

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Ho et al.[69]	2020	1,678 adolescents from Taiwan Youth Project; participants who had ever smoked cigarettes or consumed alcohol before Year 1 were excluded	*M* age = 13; 50% female; 8% Hakka, 12% Mainlanders, 1% Original Residents, 2% Other, 88% Weinan Islanders	Longitudinal; cohort starting in 7th grade (Year 1, 2000) and assessed until age 21 (Year 9, 2009); five waves of assessment	Years	Y	N	-	-	N	Y	-	Accumulating waves of sleep duration (< 6 hours) and social jet lag (≥ 0.5, 1, or 2 hours) increased the frequency of alcohol use at age 21. Sleep disturbance and sleep duration of at least 7 or 8 hours were not predictive of alcohol use.	None tested
McGlinchey & Harvey[55]	2015	4,882 adolescents from ADD Health	Wave 2: *M* age = 16; Wave 3: *M* age = 21.8; 52% female; 5% Asian, 24% Black, 4% Native American, 59% White; 8% Hispanic/Latino	Longitudinal; data from Wave 2 (1996) and Wave 3 (2001–2002)	Years	-	-	-	Y	-	-	-	Later bedtime at Wave 2 predicted increased odds of reporting alcohol "abuse" at Wave 3.	Alcohol use differences reported across sex and racial groups, although specific differences across racial groups were not specified
Mike et al.[56]	2016	186 boys from Pitt Mother and Child Project	*M* age = 11 (at baseline); follow-up between ages 20 to 22; 45% "non-White," 55% White	Longitudinal; data from baseline (age 11), with alcohol use history assessed at age 20 and age 22	Years	-	Y	-	-	-	Y	-	Shorter sleep duration and lower sleep quality both predicted earlier alcohol use, intoxication, and repeated use.	None reported
Miller et al.[171]	2016	568 college students who had violated campus alcohol policy and had been mandated to an alcohol prevention intervention	*M* age = 19.2 (*SD* = 1.2); 28% female; 84% White	Longitudinal; data from baseline, 1-, 3-, and 5-month follow-up assessments	Months	-	-	-	-	-	Y	-	Lower baseline sleep adequacy predicted alcohol-related consequences at 1-month follow-up. A stronger association was found between drinks per week and alcohol-related consequences in individuals with lower baseline sleep adequacy.	None tested

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Miller et al.[57]	2017	829 middle school students from Rhode Island study examining risk factors for initiation/ progression of drinking	*M* age = 12.6 (*SD* = 1.02); 52% female; 15% "non-White;" 12% Hispanic/Latino	Longitudinal; ongoing prospective Web-based survey over 4-year period, with recruitment beginning in 2009; data from 6-month assessments over 2 years (Times 2–5), 3-year follow-up (Time 6), and 4-year follow-up (Time 7); two sleep assessments within first 2 years of study	Years	N	-	Y	-	-	Y	-	Both shorter sleep and greater daytime sleepiness predicted onset of full drinking, heavy episodic drinking, and alcohol-related consequences. Weekend bedtime delay predicted alcohol outcomes.	Racial identity dropped from model due to nonsignificant relation to alcohol outcomes.
Miller et al.[42]	2021	56 young adults; reporting ≥ 1 binge episode in past 30 days; also meeting diagnostic criteria for insomnia	*M* age = 22.4 (*SD* = 2.7); 75% female; 5% Black, 11% multiracial, 2% Native American/ Native Alaskan, 82% White; 4% Hispanic/Latino	Intensive longitudinal design; online survey and actigraphy for at least 7 days (average 8.52 diaries; range = 1–15 days)	Days	-	Y	-	N	Y	N	-	Better self-reported sleep efficiency predicted greater drinking the next day.	Differences based on sex were not reported; differences across racial/ethnic groups were not tested.

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Nguyen-Louie et al.[58]	2018	95 adolescents from neuroimaging study on adolescent substance use in San Diego	Time 1: *M* age = 13.4 (*SD* = 0.7); Time 2: *M* age = 15.1 (*SD* = 0.9); Time 3: *M* age = 19.8 (*SD* = 0.9); 47% female; 6% Asian, 3% Black, 17% multiracial, 5.3% other, 68% White; 20% Hispanic/Latino	Longitudinal; data from Time 1 (ages 12–14), Time 2 (~ 1.5 years after Time 1), and Time 3 (~ 2.6 years after Time 2)	Years	-	-	Y	Y	-	-	Y	More frequent erratic sleep/wake behaviors (composite variable of delayed/variable bedtime and daytime dysfunction), greater sleepiness, and greater eveningness at Time 2 generally predicted more lifetime alcohol use by Time 3. However, erratic sleep/wake behaviors predicted lower alcohol use after controlling for daytime sleepiness and chronotype. Sleep variables were also tested as mediators between Time 1 risk factors (family history of SUD, inhibitory control, and internalizing/externalizing) and Time 3 lifetime alcohol use. Only erratic sleep/wake behaviors mediated Time 1 inhibitory control on Time 3 alcohol use.	None tested
Pasch et al.[59]	2012	723 adolescents from Identifying the Determinants of Eating and Activity (IDEA) or Etiology of Childhood Obesity (ECHO) cohort studies	*M* age = 14.7 (range = 10–17); 51% female; 1% Asian, < 1% American Indian, 5% Black, < 1% Native American/Pacific Islander, 6% other, 86% White; 5% Hispanic/Latino	Longitudinal; both 2-year studies; baseline data collected in 2006–2007 (IDEA) and 2007–2008 (ECHO); follow-up data collected in 2008–2009 (IDEA) and 2009–2010 (ECHO)	Years	Y	-	-	-	-	N	-	Larger weekend bedtime delay at baseline predicted higher likelihood of alcohol use at follow-up.	Sleep differences were reported across sex and racial groups.
Pieters et al.[60]	2015	555 adolescents from the Netherlands	*M* age = 13.96 (range = 11–16); 52% female; 86% had two White parents, 10% had a mix of White and "non-White" parents, 4% had two White parents	Longitudinal; two waves: Wave 1 (early 2008) and Wave 2 (late 2008)	Months	-	-	-	-	-	-	Y	Sleep problems at Wave 1 positively predicted alcohol use at Wave 2.	None tested

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Stephenson et al.[72]	2020	3,402 participants (1,435 complete twin pairs; 36% monozygotic) from FinnTwin12, a population-based study of Finnish twins born between 1937 and 1987	Time 1: *M* age = 12; Time 2: *M* age = 14; Time 3: *M* age = 22 (range = 20–26); 57% female; no information on race/ethnicity	Longitudinal; analyses focus on predictors from waves at ages 12 and 14 predicting young adult outcomes (age 22)	Years	-	-	-	-	Y	-	-	At individual level, sleeping problems at ages 12 and 14 predicted alcohol misuse in young adulthood, but were no longer significant in co-twin comparisons.	None tested
Tavernier et al.[78]	2014	780 first-year Canadian university students identifying as being morning- or evening-type	*M* age = 19 (*SD* = .09); 72% female; 87% domestic-Canadian, remaining international students (37% Asia, 10% Caribbean, 15% European Union); race not reported	Longitudinal; 2-year study: Time 1 (baseline) and Time 2 (1-year follow-up)	Years	-	-	-	-	-	-	Y	Composite variable based on chronotype, insomnia symptoms, and sleep duration. Evening-type subgroups generally reported more alcohol use than morning-type subgroups, although evening-poor sleep and morning-poor sleep subgroups did not differ. The evening subgroups (good/moderate/poor sleep) did not differ in alcohol use.	Did not test differences across racial groups; similar distribution of morning-/evening-types based on sex
Troxel et al.[6]	2021	3,265 youth from southern California study by RAND Corporation	Wave 6: *M* age = 16.2 (*SD* = 0.7); Wave 11: *M* age = 21.6 (*SD* = 0.8); 53% female; 20% Asian; 2% Black (non-Hispanic); 12% other/multiracial; 20% White (non-Hispanic); 47% Hispanic/Latino	Longitudinal; data from six waves: Wave 6 (May 2013 to April 2014) to Wave 11 (July 2018 to June 2019)	Years	Y	Y	-	Y	-	N	-	Greater trouble sleeping, later weekday and weekend bedtimes, and smaller reductions in social jet lag were associated with higher likelihood of alcohol use over time.	Differences were reported in the associations between weekday/weekend bedtime and alcohol use based on sex.
Troxel et al.[68]	2022	2,995 youth from southern California study by RAND Corporation	Wave 8: *M* age = 18.3 (*SD* = 0.8); Wave 13: *M* age = 23.6 (*SD* = 0.8); 54% female; 20% Asian, 2% Black (non-Hispanic), 12% other/multiracial, 20% White (non-Hispanic); 46% Hispanic/Latino	Longitudinal; data from six waves: Wave 8 (June 2015 to May 2016) to Wave 13 (July 2020 to July 2021)	Years	-	-	-	-	-	-	Y	"Good sleepers" (composite variable based on sleep quality, duration, and social jet lag) reported lower levels of alcohol use and consequences. When compared to "suboptimal sleepers," "good sleepers" also reported less of an increase in alcohol consequences over time.	Differences were reported in composition of classes in terms of sex and ethnicity.

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Waddell & Sasser[24]	2022	4,347 participants from ADD Health	Wave 2: *M* age = 16.0 (*SD* = 1.4); Wave 3: *M* age = 21.4 (*SD* = 1.4); Wave 4: *M* age = 28.5 (*SD* = 1.4); 52% female; 4% American Indian/Alaskan Native, 4% Asian, 24% Black, 6% other/multiracial, 68% White; 12% Hispanic/Latino	Longitudinal; data from three waves: Wave 2 (1996), Wave 3 (2001–2002), Wave 4 (2007–2008)	Years	-	-	-	-	-	Y	-	Longer sleep duration was associated with lighter drinking at the between-person level. Increases in sleep duration were associated with decreases in drinking at the within-person level. At the between-person level, higher levels of lack of premeditation were associated with greater drinking. At the within-person level, increases in sensation seeking were most strongly associated with increases in drinking for those reporting decreases in sleep duration.	None tested
Wong et al.[61]	2004	257 boys from Michigan Longitudinal Study (MLS); 60% of participants had parent with lifetime AUD	Wave 1: Age range = 3–5; Wave 2: Age range = 6–8; Wave 3: Age range = 9–11; Wave 4: Age range = 12–14; 100% White	Longitudinal; data from four regular waves at 3–year intervals	Years	-	Y	Y	-	-	-	Y	Composite index of sleep problems predicted higher likelihood of drinking at ages 12–14. Weaker associations were found for individual sleep items (trouble sleeping and overtiredness), although both predicted onset of drinking, but not drunkenness.	Not applicable
Wong et al.[63]	2009	386 children from MLS; 75% of participants with parent who met lifetime AUD	Wave 1: Age range = 3–5;* Wave 2: Age range = 6–8; Wave 3: Age range = 9–11; Wave 4: Age range = 12–14; Wave 5: Age range = 15–17; 24% female; 100% White **Note*: Girls joined study between the ages of 6 and 11.	Longitudinal; data from five regular waves and seven annual waves at 3-year intervals	Years	-	-	-	-	-	-	Y	Sleep problems during Waves 1 and 2 increased the probability of onset of alcohol use between the ages of 8 and 14 in boys but did not predict onset of alcohol use in girls before the age of 14. In contrast, sleep problems during Waves 1 and 2 increased the probability of onset of alcohol use between the ages of 15 and 17 in girls, but not boys.	Differences reported in sleep and alcohol outcomes based on sex; differences across racial groups could not be tested

Table 1. Summary of Longitudinal Studies Including Sleep-Related Predictors and Alcohol-Related Outcomes in Adolescent and/or Young Adult Samples (Continued)

		Sample		Study Design		Multidimensional Sleep Variables—Related to Alcohol Outcome(s)?								
Author	Year	Sample Size & General Description	Sample Demographics*	Design	Time Frame	Regularity	Satisfaction†	Alertness	Timing	Efficiency	Duration	Composite	Alcohol Finding	Tested Sex/Gender and/or Race/Ethnicity
Wong et al.[62]	2010	386 children from MLS; 75% of participants with parent who met lifetime AUD	Wave 1: Age range = 3–5;* Wave 2: Age range = 6–8; Wave 3: Age range = 9–11; Wave 4: Age range = 12–14; Wave 5: Age range = 15–17; 24% female; 100% White **Note*: Girls joined study between the ages of 6 and 11.	Longitudinal; data from five regular waves and seven annual waves at 3–year intervals	Years	-	N	Y	-	-	-	-	Overtiredness at ages 3–8 predicted all four alcohol variables (i.e., binge drinking, blackouts, driving under the influence, and alcohol problems) in emerging adulthood (ages 18–20). Sleep during adolescence (age 11–17) did not predict any alcohol outcomes during emerging adulthood.	Sleep differences tested by sex, though none were significant
Wong et al.[172]	2015	6,504 adolescents and young adults from ADD Health	Time 1: *M* age = 16.0 (*SD* = 1.8); Time 2: *M* age = 16.0 (*SD* = 1.6); Time 3: *M* age = 21.8 (*SD* = 1.8); Race/ethnicity/sex not reported	Longitudinal; data from three time points: Time 1 (1994–1995), Time 2 (1996), and Time 3 (2001–2002)	Years	-	Y	-	-	-	Y	-	Sleep duration at Time 1 was negatively associated with binge drinking at Time 2 (a 1-hour increase in sleep was associated with a 9% decrease in the odds of binge drinking). Sleep difficulties at Time 1 were associated with regretted sexual activities due to drinking at Time 2.	Differences reported in alcohol outcomes across sex and racial groups
Zhang et al.[65]	2011	1,611 children from Hong Kong study concerning childhood sleep problems	*M* age (baseline) = 9.0 (*SD* = 1.8); *M* age (follow-up) = 13.7 (*SD* = 1.8); 51% female; racial breakdown not specified	Longitudinal; 5-year study beginning in 2003–2004 (baseline), with follow-up assessment conducted between 2008 and 2010	Years	-	Y	-	-	-	-	-	New incidence of chronic insomnia was associated with increased risk of frequent alcohol use at 5-year follow-up, but baseline chronic insomnia and persistent chronic insomnia were not statistically significant.	Sleep differences reported by sex; differences across racial groups could not be tested.

*Age was rounded to 1 decimal place; demographic (sex/gender and race/ethnicity) percentages were rounded to whole numbers. No studies clarified whether assigned sex at birth or gender identity was assessed and reported. Therefore, the language (e.g., sex vs. gender) in the original article was retained. When possible, race/ethnicity terms were standardized to be consistent with National Institutes of Health categories. In some cases, the published papers did not specify the racial/ethnic identities beyond "White" and "non-White" (or "Caucasian" and "non-Caucasian," for which "White" and "non-White" were substituted).

†Sleep variables based on insomnia symptoms without numerical data to calculate efficiency were categorized under "Satisfaction."

Note: ADD Health, National Longitudinal Study of Adolescent to Adult Health; AUD, alcohol use disorder; ECHO, Etiology of Childhood Obesity studies; IDEA, Identifying the Determinants of Eating and Activity; *M*, mean; MLS, Michigan Longitudinal Study; NCANDA, National Consortium on Alcohol and Neurodevelopment in Adolescence; *SD*, standard deviation; SUD, substance use disorder.

A majority of the articles[5,6,51-63] also reported on other substance outcomes, particularly use of nicotine/tobacco and cannabis/marijuana, with findings suggesting that sleep-related risk for substance use may not be specific to alcohol. Indeed, the overall literature suggests a transdiagnostic scenario where multiple aspects of sleep/circadian disturbance (e.g., insomnia, sleep loss, delayed phase) increase the risk for alcohol and other substance use disorders as well as for other psychiatric disorders.[64]

Although this review focuses primarily on the period of adolescence through young adulthood, two papers based on the Michigan Longitudinal Study[61,62] and one paper based on a study in Hong Kong[65] reported that childhood sleep problems predicted later substance use, indicating that relationships between sleep and substance use are not specific to adolescents. Notably, childhood sleep tends to predict adolescent sleep,[62,65] which could partially explain the association with adolescent substance use, but also suggests the potential value of starting early with sleep-focused prevention and/or intervention efforts. Indeed, one study reported prospective sleep–substance use associations entirely within the fourth through sixth grades, and implicated inhibitory control as a potential mediator.[66] Although that study's findings contrasted with one of the papers from the Michigan Longitudinal Study (which did not support inhibitory control as a mediator in the sleep–alcohol associations),[62] changes in mood regulation, impulsivity, and/or poor decision-making remain plausible mechanisms in the longitudinal associations between childhood sleep problems and later substance use.

Several caveats are important to consider when interpreting the existing literature. First, multiple articles relied on the same longitudinal datasets; thus, 14 out of 27 longitudinal papers were based on six studies. Second, earlier studies tended to focus on only one or two sleep characteristics and were thus unable to treat sleep as a multidimensional construct. Third, papers based on more recent studies, seemingly designed to specifically consider sleep, were more likely to employ a multidimensional sleep framework.[5,6,41,53,67-69] Fourth, except for two intensive longitudinal studies[41,42] that used actigraphy—a wearable device containing an accelerometer to measure rest-activity patterns—most studies relied on self-reported sleep and are subject to the relevant biases. For example, beyond typical retrospective biases associated with self-report, there are also longstanding observations of subjective-objective discrepancies in sleep, particularly in individuals with insomnia disorder.[70] Also, none of the studies included objective circadian predictors (e.g., dim light melatonin onset) despite cross-sectional evidence that circadian timing is related to alcohol outcomes.[49,71] Fifth, observational designs cannot assess causation and directionality and therefore must be interpreted with caution. Relatedly, one recent co-twin study indicated that sleep-related risk for alcohol misuse exists over and above genetic and environmental factors.[72] However, other emerging research using genetic methods has yielded more mixed results whether the relationships between sleep/circadian characteristics and substance use should be attributed to shared genetic variance or pleiotropy[73-75] or suggests a causal relationship from sleep to substance misuse.[76]

Some of the included studies tested putative mediators of the sleep–alcohol relationship, such as behavioral inhibition, attention problems, and internalizing/externalizing symptoms; however, the results have been inconsistent (see below for further discussion). Furthermore, given that a tendency toward relatively late timing of the sleep-wake schedule (i.e., a later chronotype) is often associated with worse sleep among adolescents and young adults,[77] sleep characteristics are a putative mediator of the association between chronotype and alcohol-related risk. However, existing studies often have not supported this for alcohol[78] or other outcomes such as depression.[79,80] One study in late adolescents and young adult veterans reported that insomnia severity statistically mediated the association between depression or symptoms of post-traumatic stress disorder and alcohol use and related consequences.[81]

The time frame of assessment varied substantively across the studies, with intensive longitudinal designs narrowing the focus to day-to-day relationships whereas the more traditional longitudinal studies ranged from months to multiple years between assessments. These varying time frames are important when considering that distinct mechanistic pathways may be operating within different timescales. For example, studies with annual or multiannual time points may be speaking more to the cumulative effects of sleep/circadian characteristics, although few studies have directly tested this.[65] Interestingly, the intensive longitudinal designs (e.g., ecological momentary assessment [EMA]) appear more likely to find more nuanced associations between sleep and alcohol. For example, some EMA evidence from young adult samples suggests that better sleep efficiency[42] or quality[41] on a given night predicts more alcohol use the following day, although those findings emerged from samples with participants with sleep problems who consume alcohol. EMA findings from a much wider age range (ages 20 to 73) suggest that age may moderate sleep–alcohol associations; the younger group (age < 49) showed associations between worse sleep quality and more subsequent alcohol use whereas the older group (age > 50) drank more following nights of better sleep quality.[82]

The complex findings in EMA studies speak to the relevance of considering the multidimensional nature of sleep. In one study of undergraduate students who consumed alcohol (mean age = 20.5 years), shorter sleep and earlier wake times (based on actigraphy) and better sleep quality (based on self-report) all predicted more alcohol use the next day.[41] In the combined model that included all the sleep predictors simultaneously, only waking earlier and better perceived sleep quality upon waking predicted more alcohol use. One interpretation of

this is that shortened sleep led to deeper, more consolidated sleep, perceived in turn as higher quality, although it remains possible that shorter sleep may have impacted other intervening mechanisms (e.g., impaired cognitive control). Alternatively, as the authors suggested, late adolescents and young adults may be more likely to socialize and drink when feeling refreshed, especially given that drinking among adolescents and young adults primarily occurs in social contexts.[83] Collectively, these findings suggest the value of considering multidimensional sleep relationships with alcohol using designs that allow consideration of both short-term (i.e., day-to-day) and longer-term (i.e., months-to-years) timescales, such as embedding an EMA burst design within a longitudinal study, as done by Graupensperger and colleagues.[51] Relatedly, such designs allow parsing of between-person and within-person effects, which may well reveal distinct sleep–alcohol associations at the between-person and within-person levels.

In summary, the published longitudinal data indicate that multiple sleep and/or circadian characteristics prospectively predict alcohol-related outcomes during adolescence through young adulthood. However, the current literature is limited by overreliance on a relatively small number of longitudinal studies, largely relying on self-report measures, and insufficient consideration of the multidimensional nature of sleep. Important next steps include, but are not limited to, consideration of different timescales, including within the same study design, and examination of key mediators and moderators of sleep–alcohol associations.

Experimental Sleep and Alcohol Studies

At present, experimental evidence of causal effects of sleep on alcohol-related outcomes is based solely on insomnia treatment studies in individuals with heavy alcohol use and/or AUD, most of which are from samples older than adolescents or young adults. A systematic review and meta-analysis of nine studies of primarily middle-aged adults[7] concluded that insomnia treatment, particularly behavioral treatment, improved sleep quality and reduced depression in individuals with AUD. The authors found no definitive benefit of insomnia treatment for reducing alcohol use, although the relapse rates in two trials of cognitive-behavioral therapy for insomnia (CBT-I) were considerably lower (11% and 15%) than might be expected for adults in AUD treatment.[84] Caution is warranted in drawing strong conclusions about the potential impact on alcohol-related outcomes based on these studies, however, as the review also noted limitations related to small samples, relatively short follow-up periods, and not focusing on participants who were concurrently engaged in AUD treatment. These limitations reflect the fact that the studies generally were designed to focus on sleep outcomes rather than alcohol outcomes. Moreover, these studies varied in whether the patients were seeking or engaged in AUD treatment, and whether they were required to be abstinent at study start.

The limited published data from two sleep treatment studies in late adolescents and emerging adults are broadly consistent with the prior literature in adults, suggesting that sleep disturbance in the context of heavy alcohol use is amenable to nonpharmacological interventions; however, it remains unclear whether improving sleep measurably reduces alcohol involvement. A novel web-based intervention including both sleep and alcohol content improved sleep quality and sleep-related impairment in heavy-drinking college students,[85] although it did not outperform a control condition (psychoeducation about sleep hygiene) and did not significantly improve actigraphy-based sleep outcomes. Interestingly, although alcohol use through a 3-month follow-up declined in both conditions, reductions were larger in the control condition. The results suggested that greater reductions in sleep-related impairment may predict greater reductions in drinking (medium-to-large effect size), but those findings were not statistically significant.

Related work by Fucito and colleagues suggested that heavy-drinking college students were more receptive to sleep-focused interventions (even if they included content related to drinking) than to purely alcohol-focused interventions.[86] This may be due to less stigma associated with sleep treatment. Aside from direct effects of sleep treatment on alcohol outcomes, this could mean that sleep treatment may provide a "foot in the door" for individuals with sleep and alcohol problems. Accordingly, Fucito and colleagues are currently conducting a sleep intervention trial that focuses on sleep hygiene in young adults ages 18 to 25 who drink heavily.[87]

A more recent study tested the efficacy of CBT-I in 56 young adults ages 18 to 30 who reported monthly binge drinking and met criteria for insomnia disorder.[88,89] The study differed from prior CBT-I and alcohol studies in the sample age and that participants were still actively drinking at the start of CBT-I. The only alcohol-related treatment component was the standard sleep hygiene recommendation to reduce alcohol use before bedtime. With regard to sleep outcomes, CBT-I reduced self-reported insomnia severity relatively better than the sleep hygiene control condition, although neither treatment significantly improved actigraphy-based sleep efficiency.[88,89] Although drinking quantity and drinking-related consequences both decreased over time, these outcomes were not differentially better during CBT-I.[88] However, although insomnia improvements were not related to changes in drinking, they did mediate the reduction in alcohol-related consequences in the CBT-I group. A secondary analysis reported greater (albeit modest) reductions in alcohol craving for the CBT-I group than for the control group that, again, were statistically mediated by improvements in insomnia.[89] However, those

reductions in alcohol craving were not sustained at the 1-month follow-up assessment.

In summary, the existing experimental literature on sleep predictors of alcohol outcomes during adolescence and young adulthood is confined to a handful of trials testing nonpharmacological sleep interventions in individuals reporting heavy drinking and/or AUD. Consistent with the parallel literature in adult samples, such interventions appear beneficial for sleep-related outcomes but with no clear impact on alcohol-related outcomes. However, a preliminary finding of CBT-I reducing alcohol craving is worth further investigation, as is the further development of sleep-focused treatments, perhaps including more consideration of circadian factors.

Potential Mediators and Moderators

Prior reviews have examined plausible mechanisms linking sleep/circadian disturbances to alcohol use and alcohol-related problems, with a particular emphasis on reward function.[90-92] A recent review by the authors[91] proposed a broader conceptual model that considered both positive and negative reinforcement pathways, and noted that elevated impulsivity may exacerbate either pathway. While this model may have heuristic value, it is not without limitations. These include not explicitly addressing bidirectional effects (i.e., alcohol effects on sleep/circadian function) or incorporating plausible factors that influence which pathway is most salient for a particular individual or at a given time. Further, research on sleep–alcohol associations has largely been conducted with samples of predominantly White-identifying individuals and has largely not explored possible differences in associations between sleep and alcohol across assigned sex, racial and ethnic identities, and for LGBTQIA+ individuals. The following sections offer some preliminary evidence of the importance of including diverse samples in future investigations and of examining differences in associations to ensure generalizability of future treatments and to inform culturally responsive interventions for both sleep/circadian disturbances and AUD.

Mechanisms related to positive reinforcement

Extensive cross-sectional, longitudinal, and experimental evidence from both human and preclinical studies has supported the influence of sleep/circadian factors on reward-related processes and underlying physiology[92] and, in turn, the relevance of reward-related processes to risk for alcohol use and related problems.[28,93,94]

Although relevant human experimental studies probing sleep/circadian effects on reward-related processes have been more scarce than animal models, experimental sleep deprivation protocols have demonstrated causal effects on reward-related brain function in healthy adolescent and young adult samples.[95-97] For example, experimentally imposed circadian misalignment reduced the neural response to monetary reward and during response inhibition in healthy adolescents without regular substance use.[98] The analyses included objective measures of sleep duration and alertness, thus suggesting circadian effects on reward function beyond those of insufficient sleep. However, these studies have focused on non-alcohol rewards. In contrast to emerging animal research suggesting circadian misalignment during adolescence alters reward circuitry function and increases alcohol use during adulthood,[50] almost no published human studies have examined sleep/circadian effects on alcohol cue reactivity and/or its neural correlates. Furthermore, few existing studies have combined sleep/circadian effects, reward, and alcohol outcomes, although one cross-sectional analysis found that "eveningness"—the self-reported preference for relatively later timing of sleep and activity—was associated with altered neural processing of reward, which in turn is associated with greater alcohol use and AUD symptoms.[99] A longitudinal analysis in the same study found that the prospective association between eveningness and AUD symptoms was statistically mediated by the medial prefrontal cortex response to monetary reward.[52] Most recently, a study reported that an objective measure of circadian misalignment (measured on a Thursday) prospectively predicted a lower neural response to monetary reward (measured on a Friday) in late adolescents with regular alcohol use.[100] However, the reduced neural response to reward did not prospectively predict alcohol use that weekend, but rather was associated with more binge drinking episodes at baseline. Finally, in the aforementioned CBT-I trial in adolescents and young adults reporting heavy alcohol use and insomnia, the investigators found evidence of relatively larger reductions in delay discounting (large rewards only) in the CBT-I group, although this was not mediated by insomnia severity. However, there was no apparent effect on negative affect, suggesting that improved sleep may have relatively greater effects on reward-related processes.[89]

Some evidence suggests sleep/circadian modulation of the stimulating effects of alcohol (e.g., increases in energy and excitement). This may be particularly relevant during adolescence, when alcohol may be relatively more stimulating and less sedating than in adulthood.[101-103] Notably, a relatively more stimulating response to alcohol is a risk factor for AUD. Thus, adolescents at high risk for AUD endorsed greater alcohol-induced stimulation and stronger wanting for alcohol compared to adolescents at low risk for AUD.[104] Moreover, young adults reporting greater stimulation after alcohol administration were more likely to have developed AUD by 10-year follow-up.[105] In laboratory-based sleep studies in late adolescents and emerging adults, acute alcohol administration did not reduce SOL,[36] especially when consumed in the evening,[106] suggesting the stimulating rather than sedating effects also may be influenced by time of administration. Furthermore, later sleep timing was associated with greater self-reported stimulation response

following alcohol administration in the laboratory (at least in White male participants).[107]

Lastly, sleep/circadian factors may be relevant to positive reinforcement-related alcohol cognitions. Adolescents and young adults tend to report more motives attributed to improving their social experiences and enhancing enjoyment versus motives related to attenuating negative affect (i.e., coping).[108] Given that eveningness is associated with increased alcohol motives across the board,[109] including enhancement and social motives, it is possible that the tendency toward later sleep/circadian timing in this age group contributes to reasons for using alcohol.

Mechanisms related to negative reinforcement

Adverse life events and stress levels disrupt sleep and prospectively predict AUD outcomes, both on a longitudinal basis during adolescence into adulthood,[110,111] and more proximally (day to day).[112,113] Furthermore, demonstrating sleep- or drinking-related reactivity to stress heralds the risk for sleep-[114] or alcohol-related problems[115] in the future.

Several lines of evidence indicate that sleep problems, perhaps driven by stress and/or anxiety, may lead to using alcohol as a coping method, thus implicating negative reinforcement pathways. Studies suggest that about 10% (range 6% to 16%) of adolescents and young adults report using alcohol as a sleep aid, with higher rates in individuals with heavier alcohol use and/or worse sleep.[116-118] Interestingly, one longitudinal study of adolescents with and without AUD found that their use of alcohol as a sleep aid declined over time, dropping by half from baseline to 5-year follow-up; this may reflect adolescents' learning that alcohol's effectiveness at promoting sleep declines with regular use.[119]

Compared to "good sleepers," adults with insomnia may experience relatively greater tension reduction and deeper sleep (based on slow-wave sleep) in response to alcohol, underscoring why they might initially turn to alcohol as a sleep aid. Although experimental evidence suggests they rapidly develop tolerance to these effects, these individuals often persist in choosing alcohol as a sleep aid.[120,121] Similarly, young adults with insomnia who regularly use alcohol reported better sleep efficiency on drinking days, seemingly due to shorter SOL, in a recent EMA study,[42] and reported sleeping worse on nights when they avoided alcohol in the 2 hours before bed.[122] In contrast with the experimental study,[120] the association with better sleep efficiency remained even after accounting for number of consecutive drinking days.[42] Notably, these associations were not observed for actigraphy-based sleep efficiency.

Sleep also may modulate effects of stress on alcohol use. Along with associations with drinking motives in general (see above), eveningness in college students was associated with worse coping with stress, which in turn may predict drinking to cope.[109] Another study found that late chronotypes had both more adverse childhood experiences and greater alcohol use during young adulthood.[123]

Craving-related mechanisms

Craving—a criterion for diagnosis of AUD and widely studied as a proximal predictor of alcohol use—is a complex construct, with apparent contributions of both positive- and negative-reinforcement processes.[124] Recent studies have offered preliminary evidence that alcohol craving is influenced by sleep/circadian factors. Two studies reported the presence of a 24-hour rhythm in alcohol craving,[125,126] suggesting modulation by circadian rhythms, although the studies were mixed in whether sleep characteristics predicted the timing or amplitude of the craving rhythm. Lower sleep quality was associated with elevated tonic (i.e., long-term) craving as determined using the Obsessive-Compulsive Drinking Scale, but not with cue-induced craving (as measured using the Alcohol Urge Questionnaire) during a cue reactivity paradigm in patients with AUD.[127] Finally, less sleep predicted more alcohol craving the next day in an EMA study,[51] and reductions in insomnia severity mediated reductions in alcohol craving in a CBT-I trial.[89]

Relatedly, growing evidence implicates a role for the orexin/hypocretin system in sleep-alcohol associations via both negative reinforcement and reward-related processes. Orexin/hypocretin regulates wakefulness, reward seeking, and other motivated behavior, including alcohol craving and alcohol seeking; in turn, the orexin/hypocretin system is modulated by acute and chronic stress.[128,129] Ongoing trials are testing whether suvorexant, a dual orexin receptor antagonist, can reduce both alcohol craving and insomnia symptoms.[130,131]

Impulsivity-related mechanisms

Similar to craving, the multifaceted construct of "impulsivity" may be relevant to both positive and negative reinforcement pathways in understanding sleep/circadian-related risk for alcohol involvement. In general, facets of impulsivity are considered a key risk factor for the development of heavy alcohol use and related problems.[29,32] Importantly, impulsivity facets may differentially relate to alcohol use through both positive and negative reinforcement pathways. For example, negative urgency, or acting rashly in response to strong negative mood, may reflect drinking to cope with negative mood/stress whereas positive urgency may reflect expecting alcohol to increase arousal.[132]

Multiple sleep/circadian characteristics have been linked to impulsivity domains (e.g., Kang et al.[34,35]). For example, recent prospective evidence in adolescents suggested that both sleep duration and insomnia were bidirectionally associated with impulse control.[133] Recent studies found that later chronotype was associated with greater impulsivity overall (e.g., Kang et al.[34]), including greater self-reported trait- and state-level impulsivity across multiple subdimensions in White male

drinkers.[134] Also, as noted above, experimentally imposed circadian misalignment reduced neural activation in the right inferior frontal gyrus during response inhibition in healthy and non–substance-using adolescents.[98]

Moderation by assigned sex and gender identity

Studies found that both sleep/circadian characteristics and risk for problematic alcohol use vary by assigned sex at birth (sex); however, there has been insufficient attention to the role of sex in sleep/circadian–alcohol associations. This is important as rates of AUD among female individuals have risen 84% in the past decade, compared to a 34% increase among male individuals.[135,136] Consistent with this trend, alcohol use has risen for women but not men.[137] Prior research found that female individuals reported higher levels of disturbed sleep (e.g., insomnia),[138] while male individuals tended to report later sleeping times.[139] Recent findings suggest that sleep/circadian characteristics differentially contribute to alcohol risk for male and female individuals. Indeed, recent longitudinal studies found that male individuals in particular may be at heightened alcohol-related risk attributed to sleeplessness[138,140] and later weekday/weekend bedtime.[6] However, other studies observed stronger associations between multiple sleep characteristics (e.g., total sleep time, sleep efficiency, nighttime awakenings) and alcohol-related risks among female individuals.[5,141] Factors that may contribute to increases in alcohol use and sleep disturbance among female individuals may include heightened drinking to cope with negative affect and stress.[142-144] However, these studies did not clarify whether they were measuring assigned sex or gender identity (the term "identity" is used to reflect that race and gender are social constructs[145] and that the vast majority of research on humans asks participants to self-identify their race and gender).

Inequities in sleep[146,147] and alcohol use[148] exist for individuals with minoritized gender identities (e.g., transgender, nonbinary, gender-fluid). Importantly, a recent study examining factors that influenced sleep among individuals who identified as transgender found that one-third of the sample endorsed feelings of internalized shame (i.e., distress, anxiety, and dysphoria attributed to their identity) as reason for sleep disturbance.[149]

Inequities in sleep duration[150] and alcohol use[151] also exist among individuals with minoritized sexual orientations (e.g., lesbian, gay, queer, bisexual). However, only one cross-sectional study has examined whether sleep/circadian characteristics contribute to inequities in alcohol problems and whether these associations present differently among subgroups of people with minoritized sexual orientations (e.g., bisexual women, gay men).[152] The study found that compared to heterosexual men, gay men were less likely to experience short sleep duration and reported consuming fewer alcohol drinks per day. Lesbian and bisexual women, when compared to heterosexual women, reported a greater number of alcoholic drinks per day and were more likely to use sleep medication. Further, bisexual women were more likely to experience short sleep duration and to be diagnosed with a sleep disorder compared to heterosexual women.

It is important to place these findings within a minority stress model framework, where individuals with minoritized identities are exposed to identity-based stressors[153] that occur at both interpersonal and systemic levels.[154] Identity-based stressors—defined as chronic modes of stress attributed to discrimination and internalized stigma directed at one's minoritized identity (e.g., sexual, gender, or racial identities)—are prominent predictors of health inequities, including alcohol behaviors and sleep disturbances, among individuals with minoritized sexual and gender identities.[146,155,156] However, further examination of possible differential associations between sleep indices and alcohol behaviors is needed.

Moderation by racial and ethnic identities

As a function of sociohistorical context and multiple levels of discrimination, inequities in sleep health and alcohol problems have been shown for individuals with minoritized racial and ethnic identities.[157-162] Significantly less research has examined if sleep disturbances related to discrimination contribute to the inequities in alcohol problems and whether the associations between sleep and alcohol differ among individuals with different racial or ethnic identities. Structural racism affects neighborhood-level factors that impact sleep (e.g., noise pollution) and alcohol use (e.g., alcohol outlet density), and neighborhood socioeconomic indicators (i.e., income, crime rates, discrimination) have been implicated in inequities in sleep, which may contribute to downstream poor health outcomes.[163] Specifically, studies have identified that individuals with low socioeconomic status tend to inhabit urban areas, which may be more hazardous and noisier and may have higher levels of crime. Such neighborhood characteristics have been found to be associated with greater rates of chronic sleep disturbance,[164] which in turn have been linked to heightened alcohol consumption among adolescents as reviewed above (also see Edwards, Reeves, and Fishbein[165]). As individuals with minoritized racial and ethnic identities may be more socioeconomically disadvantaged as a result of sociohistorical structural and interpersonal discrimination, these youth may be at greater risk for poor sleep quality in addition to elevated risk for alcohol use. These environmental factors may also affect associations between sleep and alcohol differently for individuals with minoritized racial or ethnic identities. All of these potential associations have direct implications for prevention and treatment.

Cross-sectional evidence suggests that alcohol use may be more disruptive to sleep for Black individuals relative to White individuals. Among men with AUD, Black men had more severe

sleep disturbances compared to White men.[166] Based on National Health Interview survey data collected between 2004 and 2015, sleep duration and sleep quality were highest in Black individuals who never consumed alcohol (i.e., lifetime abstention) and worsened as alcohol use involvement increased.[167] For White individuals, this pattern was more variable. Importantly, the racial differences in this study were more pronounced for women than men, demonstrating the importance of examining intersectionality.

Research examining associations between sleep and alcohol use in minoritized racial or ethnic groups beyond Black or African American individuals is nascent. However, consistent with research with predominantly White samples, binge drinking in adolescence has been shown to relate to poorer sleep quality in young adulthood for Mexican American and American Indian (as defined in the article) individuals.[168]

Studies examining how sleep may differentially affect alcohol use and experiences while drinking across racial and ethnic groups are even more sparse. Preliminary research found that later sleep timing was related to increased sensitivity to the stimulating effects of alcohol for White men but not Black men;[107] however, no differences existed in associations with 24-hour rhythms in alcohol craving for Black and White young adults.[125]

Other possible moderators

Multiple other moderators of the relationship between sleep/circadian factors and alcohol use are plausible but have received little attention to date, including the role of age and/or developmental stage. An exploratory analysis of the longitudinal data from the National Consortium on Alcohol and Neurodevelopment in Adolescence study[5] found a different pattern of sleep/circadian predictors of binge alcohol severity at middle- and high-school age time points versus post–high-school age time points. This difference could reflect context, given systematic early school start times versus more flexibility in schedules after high school (i.e., college and/or employment), but more research is needed to replicate and further clarify this finding.

Sleep/circadian-related risk for alcohol outcomes also may be moderated by the stage of alcohol use and related problems, potentially varying as individuals progress through the three-stage cycle framework of AUD—binge/intoxication, negative affect/withdrawal, and preoccupation/anticipation as described by Koob and Colrain.[2] The shift from enhancement motives/positive reinforcement in the binge/intoxication phase to coping motives/negative reinforcement in the withdrawal/negative affect stage could be paralleled by a shift in relevant sleep/circadian pathways. That is, accumulating alcohol use/problems may contribute to more chronic and/or more distinct sleep/circadian disturbances, which in turn may maintain or exacerbate alcohol involvement. Additionally, sleep problems have been identified as a risk factor for relapse during early abstinence in individuals with AUD.[2]

Conclusions and Future Directions

Based on the above discussion, future research on the intersection between sleep and alcohol should address existing gaps related to both research methodology and specific questions addressed. For example, future studies should employ assessment batteries able to assess multidimensional sleep/circadian characteristics and should include both self-report and objective measures, particularly objective assessments not yet sufficiently leveraged in this literature, such as the Multiple Sleep Latency Test to assess daytime sleepiness. Research also can benefit from the use of combined longitudinal and intensive longitudinal designs, such as EMA bursts within a larger longitudinal study framework, which will allow consideration of both different timescales and parsing of between-person (trait) and within-person (state) effects.

Such studies should further explore the role of relevant moderators, with particular attention to sleep–alcohol associations for individuals with minoritized identities. Equally important is consideration of the association between sleep and cannabis use, including simultaneous use with alcohol, given the high prevalence of this practice in late adolescents and young adults and evidence suggesting somewhat opposing effects of both substances on sleep. Examination of potential differences in sleep–alcohol associations across international samples could help determine how varying cultural contexts may differentially influence sleep, alcohol use, and their association.

Furthermore, experimental research is needed to demonstrate causal effects of sleep/circadian manipulations on alcohol-related risk. Additionally, experimental studies using approaches such as forced desynchrony or ultradian sleep-wake protocols could help parse the role of circadian versus sleep homeostatic contributions in modulating alcohol-related processes (e.g., alcohol craving).

Other research gaps to be addressed include the clarification of potential shared genetic variance and/or pleiotropic contributions to sleep–alcohol associations, which should further clarify trait- versus state-level effects, as well as investigation of different mechanistic pathways linking sleep to alcohol outcomes. These ideally should allow for comparison of distinct pathways within the same dataset and include not only the putative mechanisms described above (e.g., reward function, negative reinforcement, impulsivity) but also others that may well be worth consideration, such as hypothalamic-pituitary-adrenal axis function.

Finally, research gaps exist with respect to treatment of adolescents and young adults with both alcohol problems and

sleep problems. Rigorous treatment studies in this population are needed that go beyond CBT-I to include attention to circadian factors, and with sufficient follow-up periods to better elucidate differential effects on alcohol.

Overall, the existing longitudinal and experimental evidence indicates that a range of sleep/circadian characteristics during adolescence and young adulthood influence risk for the development of alcohol use and/or related problems. Although studies in late adolescents and young adults engaging in regular and/or heavy drinking show that sleep treatment can improve sleep in those individuals, as well as potentially reduce alcohol craving and alcohol-related consequences, no studies in any age group have yet demonstrated that improving sleep reduces drinking behavior. Future research embedding intensive longitudinal studies within prospective research studies is needed to understand the underlying mechanistic pathways from sleep and circadian rhythm to differential alcohol use behaviors and problems as there is evidence that specific sleep indices may relate to certain AUD criteria.[169] Such studies could hold promise for informing treatment for both sleep problems and AUD.

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Gut-Liver-Brain Axis and Alcohol Use Disorder: Treatment Potential of Fecal Microbiota Transplantation

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PURPOSE: Chronic alcohol use is a major cause of liver damage and death. In the United States, multiple factors have led to low utilization of pharmacotherapy for alcohol use disorder (AUD), including lack of provider knowledge and comfort in prescribing medications for AUD. Alcohol consumption has direct effects on the gut microbiota, altering the diversity of bacteria and leading to bacterial overgrowth. Growing evidence suggests that alcohol's effects on the gut microbiome may contribute to increased alcohol consumption and progression of alcohol-associated liver disease (ALD). This article reviews human and preclinical studies investigating the role of fecal microbiota transplantation (FMT) in ameliorating alcohol-associated alterations to the liver, gut, and brain resulting in altered behavior; it also discusses the therapeutic potential of FMT.
SEARCH METHODS: For this narrative review, a literature search was conducted in September 2022 of PubMed, Web of Science Core Collection, and Google Scholar to identify studies published between January 2012 and September 2022. Search terms used included "fecal microbiota transplantation" and "alcohol."
SEARCH RESULTS: Most results of the literature search were review articles or articles on nonalcoholic fatty liver disease; these were excluded. Of the remaining empirical manuscripts, very few described clinical or preclinical studies that were directly investigating the effects of FMT on alcohol drinking or related behaviors. Ultimately, 16 studies were included in the review.
DISCUSSION AND CONCLUSIONS: The literature search identified only a few studies that were directly investigating the effect of FMT on ALD or alcohol drinking and related behaviors. Largely proof-of-concept studies, these findings demonstrate that alcohol can alter the gut microbiome and that the microbiome can be transferred between humans and rodents to alter affective behaviors frequently associated with increased alcohol use. Other studies have shown promise of FMT or other probiotic supplementation in alleviating some of the symptoms associated with ALD and drinking. These results show that the implementation of FMT as a therapeutic approach is still in the investigatory stages.

KEYWORDS: alcohol; fecal microbiota transplant; alcohol-associated liver disease; gut-brain axis; gastrointestinal microbiome; microbiota; probiotics; behavior

Alcohol-associated liver disease (ALD) is a leading cause of morbidity and mortality in people with alcohol use disorder (AUD).[1] Alcohol exerts its effect on the liver through both direct and indirect pathways and can eventually lead to steatosis, steatohepatitis, fibrosis, hepatocellular carcinoma, and cirrhosis.[2] However, only approximately 10% to 20% of patients with ALD develop cirrhosis.[2] When decompensated cirrhosis develops, liver transplantation should be considered; however, a transplant may not be a feasible option for certain patients. Transplant eligibility is determined in a multidisciplinary fashion that includes a vigorous medical, psychosocial, surgical, and financial evaluation. Furthermore, the peri- and post-transplant periods can pose unique challenges to patients with underlying AUD. Individuals with chronic AUD are at risk for nutrient deficiencies, malnourishment, and sarcopenia.[3] As such, they can enter transplant in a frail state that can predispose patients to infection, impaired wound healing, and sarcopenia (loss of muscle mass and function). In addition, transplant committees often require that patients engage in post-transplant alcohol cessation programs. To obviate the need for liver transplants, efforts to treat AUD and reduce craving should begin earlier in the disease course. In the United States, currently approved pharmacologic therapies for AUD include disulfiram, acamprosate, and naltrexone.[4]

Although pharmacological treatments exist, the treatment gap for AUD is higher than for any other mental disorder,[5] and these treatments are prescribed only for a small percentage of patients with AUD. Several factors may contribute to the underuse of pharmacologic treatments for AUD, including lack of provider knowledge and comfort in prescribing these medications, low compliance with treatment among patients, and patient heterogeneity combined with the availability of only three approved medications. Thus, most patients with AUD—especially those with advanced AUD—are left untreated, and there is a need for additional, more effective therapies.

Newer therapeutic regimens include gut microbiome manipulation, which may modulate alcohol intake and drinking behavior.[2,6] Growing evidence suggests that alteration of intestinal microbiota—which include not only bacteria but also fungi and viruses—contributes to the progression of excessive alcohol consumption and ALD, and this may form a therapeutic target.[2,6] Alcohol consumption has both direct and indirect effects on the gut microbiota via alcohol metabolism, activation of inflammatory cascades, and alterations in the enteric nervous system.[2,6] This suggests that by altering the gut microbiota, alcohol consumption may be modulated, slowing the progression of ALD.[2,6]

The Impact of Alcohol on the Gut-Liver Axis

Gut-liver communication occurs both through the hepatic portal vein and the hepatic biliary system and can be influenced by the gut microbiota.[6] Dietary nutrients absorbed from the gut can be carried directly to the liver via the portal vein. However, if the gut microbiota composition or gut barrier function is disrupted, other mediators or toxins can take the same route to disrupt liver homeostasis.[7] The hepatic biliary system along with systemic circulation allows the liver to provide feedback to the gut via release of bile acids and other bioactive molecules.[6]

Alcohol consumption induces gut dysbiosis, an imbalance in gut microbiota, through several mechanisms. Chronic alcohol exposure decreases the production of mucus and antimicrobial peptides such as alpha-defensins and disrupts the intestinal barrier.[2,8,9] This allows for translocation of lipopolysaccharide (LPS) and other endotoxins into the liver via the portal vein.[10] LPS is produced by gram-negative bacteria and is one of the main factors in the pathogenesis of ALD. LPS activates toll-like receptors on the surface of Kupffer cells and induces pro-inflammatory signaling cascades, the release of cytokines, and, ultimately, hepatocyte damage.[6] People with ALD often show higher levels of circulating pro-inflammatory mediators, such as LPS, interleukin 8 (IL-8), and IL-17.[11] Pro-inflammatory circulating cytokines were found to positively correlate with scores of depression, anxiety, and alcohol craving in active drinkers.[12] Moreover, inflammation markers were found to correlate with ALD severity.[7,13]

Alcohol use could also alter gut microbiota by reducing production of short-chain fatty acids (SCFAs), which are beneficial fermentation products.[14] SCFAs have anti-inflammatory and immune-modulatory activity and help maintain the intestinal barrier.[6] Alcohol has been shown to decrease SCFA production, reflected in the fecal content of patients with alcohol-associated cirrhosis.[15] This alcohol-induced disruption of bacterial metabolites (such as SCFAs, and bile acids among others) is a consequence of altered gut microbiota composition.

Alcohol use has been shown to result in bacterial overgrowth and dysbiosis. In general, alcohol reduces *Bacteroidetes*, *Clostridia*, and *Verrucomicrobiae* and leads to increases in *Proteobacteria*, *Gammaproteobacteria*, and *Bacilli*.[16] Alcohol also has direct cytotoxic effect on hepatocytes; its metabolite acetaldehyde triggers pro-inflammatory signaling cascades and damages the epithelial barrier.[9]

The Impact of Alcohol on the Gut-Brain Axis

The gut microbiome also influences brain function and behavior through a variety of mechanisms and thus may be involved in the onset and severity of some psychiatric disorders, such as AUD.[6] Research has suggested that bacterial metabolites can cross the blood-brain barrier via sensory nerves that innervate the gut.[6] In patients with AUD, chronic low-grade inflammation leads to changes in pro-inflammatory mediators that can cross the blood-brain barrier to activate nuclear factor kappa B (NF-kB) in glial cells, leading to neuronal damage.[17] This concept was further confirmed in a study demonstrating that a single injection of LPS led to increases in tumor necrosis factor-alpha (TNF-alpha) in the liver and brain, promoted microglial activation, and induced degeneration of dopamine-secreting neurons.[17] Although some bacterial species can produce neurotransmitters, such as gamma-aminobutyric acid (GABA) and dopamine, it is debated whether these neurotransmitters can cross the blood-brain barrier.[6] It may be that signaling by the vagal nerve influences neurotransmitter production, which could impact behaviors associated with AUD, such as anxiety.[6] However, anti-inflammatory cytokines such as IL-10 have been shown to reverse anxiety-like behavior related to substance use.[18] Thus, multiple factors can influence the development of mood disorders. Vagal signaling may play a critical role in the onset and severity of AUD, as significant reduction in voluntary drinking was seen in rats that underwent vagotomy.[19]

Microbiota-derived ammonia can also impact the central nervous system.[6] Due to poor hepatic clearance, high levels of ammonia are seen in some patients with ALD, which can reach the brain and lead to astrocyte death, brain damage, and cognitive alterations. Another potential mechanism how gut microbiota may affect brain function is through the previously discussed alcohol-related decrease in levels of SCFAs, such as butyrate.[6] Butyrate is a potent inhibitor of histone deacetylases and thus can lead to epigenetic changes such as modulation of histone modifications.[20] Such epigenetic changes in the brain have the potential to impact current and future substance use by modulating addiction and reward networks.[21] One study reported correlations between the gut microbiome and behavioral and neurophysiological traits that define AUD, such as measures of impulsivity and augmentations in striatal dopamine receptor expression.[22]

This review presents the growing number of clinical and preclinical studies that are beginning to investigate the therapeutic role and mechanisms underlying fecal microbiota transplantation (FMT) in ALD and AUD (see Table 1). It is important to note that not all patients with AUD have dysbiosis and/or increased intestinal permeability; the reason for this is unclear. A literature search using the terms "fecal microbiota transplantation" AND "alcohol" found very few studies that directly investigated the effect of FMT on alcohol drinking behavior. In addition, only a small number of articles showed the impact of FMT on affective behaviors that are frequently associated with excessive alcohol use. Some studies have shown promise in using gut microbial manipulation for alleviating some of the symptoms associated with ALD. Using these studies, the review outlines the interplay between the modulation of the gut microbiome, the gut-liver-brain axis, and AUD. The article also discusses why microbiome manipulation may be a promising therapeutic for ALD and proposes future directions.

Search Methods

A September 2022 search of the PubMed database using the search terms "fecal microbiota transplantation AND alcohol, NOT review" identified 71 articles that were published between January 1996 and September 2022. Among these articles, 16 were preclinical studies that used alcohol in their model (e.g., animals treated with alcohol, or animals treated with FMT from alcohol-exposed subjects). Most of the excluded articles described studies of non–alcohol-associated liver disease. Of the 16 included preclinical publications, six assessed the effects of FMT or the modulation of the microbiome on ALD. Six other articles investigated the role of modulation of the gut microbiome on alcohol-associated behaviors (e.g., sociability, anxiety, and depression) or drinking behavior, with some reporting changes in gene or protein expression in the brains of recipient animals. The other four articles not directly discussed below were excluded for the following reasons: one article was a commentary, and three were focused on alcohol's role on innate and adaptive immunity or pulmonary infection, not the gut-liver-brain axis. The 71 identified articles included 11 human/clinical studies, but four were excluded because they were either not related to alcohol or were not focused on microbial therapeutics. The remaining seven articles were human/clinical studies related to alcohol or cirrhosis (see Table 1).

A similar search strategy was employed in the Web of Science Core Collection database and Google Scholar. These searches identified 32 publications, and these were also contained in the PubMed dataset. Of note, none of these publications were published prior to 2016.

Table 1: Summary of Preclinical and Clinical Studies Assessing the Effects of Fecal Microbiota Transplant (FMT) on Alcohol-Related Outcomes

Study*	Subjects	Model	Main Finding
Ferrere et al. (2017)[23]	Mice	Signs of ALD lesions after Lieber-DeCarli diet	FMT prevented the development of alcohol-induced liver lesions, but the effect depended on the host microbiome.
Wrzosek et al. (2021)[30]	Mice	Signs of ALD after FMT from SAH patients	Pectin-FMT beneficially reshaped the GM, in an AhR-dependent manner.
Yu et al. (2020)[31]	Mice	Signs of ALD lesions after Lieber-DeCarli diet with ethanol	FMT or LRP6-CRISPR improved GM diversity and composition to ameliorate ALD symptoms.
Yan et al. (2021)[32]	Mice	Signs of ALD lesions after Lieber-DeCarli diet with ethanol	TQE supplementation or TQE-FMT alleviated chronic alcohol-induced liver injury and markers of gut barrier dysfunction.
Yan et al. (2021)[33]	Mice	Signs of ALD lesions after Lieber-DeCarli diet with ethanol	UA had hepatoprotective effects and suppressed alcohol-induced oxidative stress and intestinal barrier disruption.
Guo et al. (2022)[34]	Mice	Acute ALD signs by ethanol lavage	Goji berries restored intestinal epithelial cell integrity and prevented acute liver injury induced by alcohol intake.
Xiao et al. (2018)[39]	Mice	FMT from noncontingent drinking mice	Alc-FMT transferred negative affective behaviors following withdrawal, altered brain gene expression, and reduced GM diversity.
Segovia-Rodriguez et al. (2022)[40]	Rats	FMT from ethanol-exposed rats (10 g/kg for 10 days)	Alc-FMT increased drinking and reduced locomotor activity, but this was dependent on antibiotics pretreatment.
Ezquer et al. (2022)[42]	Alcohol-preferring rats	Alcohol relapse drinking and LGG treatment	LGG modified the GM, reduced alcohol intake, and altered brain protein expression in a model of relapse drinking.
Bajaj et al. (2021)[56]	Humans	Patients with alcohol-associated cirrhosis and AUD	FMT reduced alcohol consumption and cravings and increased microbial diversity.
Philips et al. (2022)[58]	Humans	SAH hepatitis patients	FMT decreased alcohol relapse rates and increased time to relapse, increased beneficial GM diversity, and lowered rates of infections and hospitalizations with higher survival rates.
Philips et al. (2017)[59]	Humans	Open-label study of patients ineligible for steroid therapy	FMT recipients had higher transplant-free survival associated with reduction in pathogenic bacteria.
Sharma et al. (2022)[60]	Humans	Open-lab nonrandomized trial with severe alcohol-associated hepatitis with ACLF	FMT significantly reduced 28- and 90-day mortality and inflammatory cytokines.
Bajaj et al. (2017)[62]	Humans	Open-label randomized trial: outpatient men with cirrhosis and recurrent HE received FMT enema	Improved cognition along with increased microbial diversity.
Bajaj et al. (2019)[65]	Humans	Randomized, single-blind study: cirrhosis with recurrent HE receiving FMT capsules vs. placebo	FMT capsules were safe and improved duodenal mucosal diversity, dysbiosis, and objective measures of encephalopathy.
Philips et al. (2018)[68]	Humans	Comparative study between pentoxifylline, corticosteroid, nutritional therapy, and FMT	FMT had highest survival rates at 3-month follow-up by modulating GM composition and function and decreasing inflammatory pathways.
Zhao et al. (2020)[38]	Humans to mice	Cross-species Alc-FMT	Human to mouse Alc-FMT increased alcohol preference and negative affective behaviors and altered brain gene expression.
Wolstenholme et al. (2022)[41]	Humans to mice	Cross-species Alc-FMT and treated Alc-FMT	Alcohol preference and intake were reduced in patients with AUD after receiving FMT, and this behavior was transmissible to mice; liver, intestine, and brain gene expression was altered in mice.
Leclercq et al. (2020)[43]	Humans to mice	Cross-species Alc-FMT	Human-to-mouse Alc-FMT increased depression-like behavior and lowered sociability; brain neurotransmitter and myelin gene expression were altered.

*Studies are ordered by citation number within each subject type.

Note: ACLF, acute-on-chronic liver failure; AhR, aryl hydrocarbon receptor; Alc, alcohol; ALD, alcohol-associated liver disease; AUD, alcohol use disorder; CRISPR, clustered regularly interspaced short palindromic repeats; FMT, fecal microbiota transplant; GM, gut microbiota; HE, hepatic encephalopathy; LGG, *Lactobacillus rhamnosus* Gorbach-Goldin; LRP6, low-density lipoprotein-related protein 6; SAH, severe alcohol-associated hepatitis; TQE, *Thymus quinquecostatus* Celak extract; UA, ursolic acid.

Results

Gut Microbiome and ALD: Preclinical Studies

In one of the seminal preclinical studies to investigate whether manipulation of the intestinal microbiome can prevent the development of ALD, Ferrere et al. showed that factors other than alcohol exposure are involved in the development of ALD.[23] In this study that compared mice raised in two different institutions and that were fed the same Lieber-DeCarli diet—a liquid diet for rodents that contains all dietary and hydration needs as well as alcohol to induce the pathogenesis of early-stage ALD—mice consumed similar amounts of alcohol, had similar liver weights, and initially had similar fecal microbiota composition. However, mice from one facility developed early signs of ALD while mice from the other facility did not. Following 10 days of the Lieber-DeCarli diet supplemented with 5% ethanol, the animals exhibited specific microbiota profiles that were associated with susceptibility or resistance to ALD symptoms. In the ALD-sensitive mice, the alcohol diet induced a decrease of cecal *Bacteroidetes* and *Proteobacteria* and an increase of *Actinobacteria* and *Firmicutes*. Thus, the ALD-sensitive mice had 50% less *Bacteroides* than did the ALD-resistant mice at the end of the 10-day period. To prove that the microbiota were likely responsible for ALD sensitivity or resistance, the researchers performed FMT by transferring fecal matter from ALD-resistant mice to ALD-sensitive mice. FMT or pectin (complex heteropolysaccharides that can modulate the growth of gut microbiota) treatment protected the susceptible mice from alcohol-induced depletion of *Bacteroides*, and the microbiomes of FMT-treated mice were similar to the microbiomes of ALD-resistant mice. Moreover, FMT prevented the development of alcohol-induced liver lesions.[23] This study was an important first step in showing that the endogenous microbiome influences an individual's susceptibility to ALD and that manipulation of the intestinal microbiome can prevent the development of alcohol-induced liver lesions and may be a strong therapeutic treatment strategy.

Following this seminal study, additional research groups investigated whether probiotics or dietary supplements that alter the microbiome can also reduce ALD symptoms.[6,19,24-29] These studies generally demonstrated a positive outcome of treatment with probiotics on liver outcomes; however, as they did not use FMT, a detailed discussion is beyond the scope of this article. To mechanistically understand how pectin alters the intestinal microbiome and therapeutically treats ALD, mice received an FMT from patients with severe alcohol-associated hepatitis to establish alcohol-induced liver lesions in the context of the human microbiota.[30] The animals were then treated with pectin via FMT. Compared with control animals, pectin-treated mice showed a higher number of bacterial genes involved in carbohydrate, lipid, and amino-acid metabolism. Metabolomic analyses identified alterations in bacterial tryptophan metabolism and increased indole derivatives, suggesting activation of the aryl hydrocarbon receptor (AhR) signaling system. AhR agonists simulated the effects of pectin in liver tissue and reversed the signs of ALD. Conversely, knock-out of the AhR gene in mice reduced the effects of beneficial microbiota on alcohol-induced liver injury. Finally, the researchers found decreased level of AhR agonists in patients with severe alcohol-associated hepatitis, suggesting that AhR may be a new therapeutic target in ALD.[30] These findings indicate that pectin reshapes the microbiome in the context of the human microbiota and not only prevents, but reverses, alcohol-induced liver injury in mice.

In another study, Yu et al. directly compared FMT to clustered interspaced short palindromic repeats (CRISPR) inactivation of low-density lipoprotein receptor-related protein 6 (LRP6), a co-receptor of the canonical Wnt/beta-catenin pathway, in their ability to ameliorate ALD symptoms.[31] Knock-down of LRP6 by CRISPR, they hypothesized, would reduce Wnt signaling in hemopoietic stem cells to reduce their activation and, thus, improve the effects of liver fibrogenesis in their model of ALD. Rats fed an ethanol-containing Lieber-DeCarli diet to induce liver fibrosis and model early-stage ALD were then administered FMT from healthy rats or treated with LRP6-CRISPR. Histological and molecular assays revealed moderately improved liver histological markers in the FMT-treated rats that were accompanied by similar changes in fibrosis biomarkers. LRP6-CRISPR–treated mice showed similar improvements in liver histology and molecular markers, but with a greater effect size. Both LRP6-CRISPR and FMT treatment partially restored the composition of the gut microbiome and increased gut microflora diversity. Compared with untreated ALD-rats, LRP6-CRISPR and FMT both increased gut microbiota richness and diversity and resulted in a similar microbiota composition structure. Thus, principal coordinate analysis indicated that the gut microbiome of rats treated with LRP6-CRISPR and FMT overlapped and intersected with each other and with the control group. Specifically, LRP6-CRISPR and FMT each increased abundance of *Lactobacillus*. Thus, targeting the gut microbiome using samples from healthy rats or directly inactivating a member of the Wnt signaling pathway can improve the diversity and composition of the microbiome to ameliorate ALD symptoms.[31]

Three studies have used FMT procedures to show that gut microbiome remodeling may be a causal mechanism underlying the hepatoprotective effects and reductions in alcohol-induced liver injury of specific dietary enhancements, such as ursolic acid (UA) or Goji berries.[32-34] UA, a bioactive constituent in teas, fruits, edible plants, and herbs, also has hepatoprotective activity.[35-36] Using a model of chronic alcohol exposure to induce liver injury, Yan et al. showed that UA had not only hepatoprotective effects, but also suppressed alcohol-induced oxidative stress and intestinal barrier disruption.[33] An FMT study was performed to investigate the possible contribution

of gut microbiota manipulation in the beneficial effects of UA on alcohol-induced liver injury. Compared to mice receiving control-FMT, recipients of FMT from UA-consuming donors had a remodeled gut microbiome, less alcohol-induced gut dysbiosis, and reduced oxidative stress.[33] Alcohol-induced liver injury was also partly alleviated in UA-FMT recipient mice, suggesting the hepatoprotective activity of UA is transferable and can be partly attributed to gut dysbiosis correction.[33] Using a traditional Chinese medicinal plant, Goji berries, Guo et al. were able to restore the intestinal epithelial cell integrity and prevent acute liver injury induced by alcohol intake in mice.[34] To examine whether the Goji-modulated gut microbiota played a causal role on liver protection, an FMT experiment was performed in mice pretreated with antibiotics. FMT from donors that consumed Goji berries also protected against elevations in markers of acute alcohol-induced liver injury in recipient mice.[34] *Thymus quinquecostatus* Celak extract (TQE) is a species of thyme, widely used as food additive in Asia, that possesses hepatoprotective activity.[37] To investigate the mechanisms of TQE's liver protective effects in vivo, TQE supplementation alleviated chronic alcohol-induced liver injury and markers of gut barrier dysfunction in mice, likely through suppression of toll-like receptor 4-mediated inflammatory response and overproduction of reactive oxygen species.[32] FMT studies using material from TQE-exposed donors also counteracted the alcohol-induced gut dysbiosis and partially ameliorated liver injury in the recipient mice, suggesting a causal role of the gut-liver axis in the hepatoprotective effects of TQE.[32] Together, these studies show hepatoprotective effects of dietary supplements on acute or chronic alcohol-induced liver disease. FMT was used to show that these hepatoprotective effects can be transferrable and show causal role of the gut-liver axis in models of ALD.

Gut Microbiome and Alcohol Consumption: Preclinical Studies

Few studies have used preclinical models to directly investigate the role of the gut microbiome on alcohol drinking or alcohol-related phenotypes such as anxiety and depression.[38-43] Some of these studies used cross-species FMT to establish causality of the gut microbiome on alcohol drinking and related behavior.[38,40-42] Most of these six studies investigated the effect of microbiomes after alcohol exposure on similar outcomes and on gene or protein expression within the brain.[38,39,41-43] In one of the first studies directly assessing the ability of the gut microbiome to contribute to the development of alcohol-related behaviors, transplantation of gut microbiota from alcohol-fed mice facilitated the development of depressive-like behavior in alcohol-naïve recipients.[39] In this model of noncontingent voluntary alcohol consumption, 4 weeks of escalating ethanol concentrations in the drinking water did not alter bacterial abundance but did change gut microbiota composition. Alcohol-exposed mice displayed signs of negative affective behavior following alcohol withdrawal in two rodent models of depression (i.e., the forced swim and tail suspension tasks). Additionally, they exhibited decreased expression of the brain-derived neurotrophic factor (*Bdnf*) and corticotropin-releasing hormone receptor 1 (*Crhr1*) genes, as well as increased expression of the mu opioid receptor (*Oprm1*) gene in the hippocampus. Fourteen days of daily FMT from alcohol-drinking mice into alcohol-naïve recipients (Alc-FMT) increased their depression-like behavior, similar to that of the alcohol-drinking donors. These findings were interpreted as transference of behavioral signs of alcohol withdrawal-induced negative affect. Additionally, similar gene expression changes in *Bdnf*, *Crhr1*, and *Oprm1* found in alcohol-exposed mice were seen in the hippocampus of Alc-FMT mice. Finally, as seen in previous studies, both alcohol consumption and alcohol-FMT decreased the relative abundance of *Lactobacillus* and increased *Allobaculum* abundance.[39]

To investigate whether changes in the gut microbiome are a cause or a consequence of alcohol drinking, Segovia-Rodriguez et al. treated alcohol-naïve rats with FMT from rats exposed to high (10 g/kg) ethanol doses (Alc-FMT), control-FMT, or phosphate-buffered saline control for 10 days.[40] Antibiotic pretreatment was also tested in each group given the known effects of antibiotics on gut microbiome diversity and alcohol intake. Alc-FMT rats without antibiotic pretreatment increased their alcohol intake as compared to rats given control buffer via oral gavage, while control-FMT mice had decreased alcohol intake in the drinking in the dark multiple scheduled access model. The increased intake in Alc-FMT rats occurred 2 weeks after the last fecal transplant. The researchers suggested that this could be due to an interaction between the new Alc-FMT microbiota received and alcohol consumption, producing a synergistic effect that favored bacteria most benefited by alcohol consumption. Antibiotic pretreatment caused a significant reduction in alcohol consumption, and neither Alc-FMT nor control-FMT had an effect on intake. Additionally, spontaneous locomotor activity was reduced in the Alc-FMT mice, and antibiotic pretreatment abolished this effect.[40] The findings suggest that, similar to the study by Ferrere et al.,[23] alcohol preference may be dependent on the content of the gut microbiome since antibiotic pretreatment abolished the effects of both control-FMT and Alc-FMT.[40]

In another study not involving FMT, a dietary probiotic (*Lactobacillus rhamnosus* Gorbach-Goldin [LGG]) was used to modify the gut microbiota and assess alcohol intake in a rat model of alcohol relapse drinking.[42] Rats selectively bred for alcohol drinking consumed alcohol for 5 weeks before they were administered antibiotics followed by daily LGG during a forced deprivation period. Antibiotic treatment alone led to a reduction (30%–40%) of early alcohol relapse drinking (i.e., within 60 minutes of restored access to alcohol), which increased to a 20% decrease of relapse drinking with 24-hour access. LGG treatment inhibited relapse drinking by 66% to

80%, as did administration of *N*-acetylcysteine + acetylsalicylic acid (NAC+ASA), which inhibits the alcohol-induced hyperglutamatergic condition. However, the combination of LGG and NAC+ASA during the deprivation period showed additive effects and virtually suppressed (90% inhibition) binge-like drinking after renewed access to alcohol. The reductions in alcohol deprivation effect were accompanied by differential alterations in protein levels in the nucleus accumbens. LGG treatment increased dopamine transporters, while NAC+ASA increased glutamate transporter levels (xCT and GLT-1), suggesting these dietary supplements are acting through different mechanisms to reduce alcohol relapse.[42]

Role of Gut Microbiome in ALD: Clinical Studies

The gut microbiome—including bacteria, fungi, and viruses—has been implicated in the progression of liver disease in patients with underlying AUD; however, the few clinical studies that exist offer variable results.

Bacteria

A study by Maccioni et al. compared patients with ALD to healthy controls in an analysis of microbiota from feces and duodenal mucosa.[44] In this study, patients with hepatic inflammation and fibrosis had increases in potentially pathogenic bacterial taxa, including *Streptococcus*, *Shuttleworthia*, and *Rothia*. This supports the notion that alcohol exposure increases intestinal permeability and that this can potentially contribute to ALD development, though further studies are warranted. Patients with alcohol-associated cirrhosis exhibit an increase in oral microbial species (*Lactobacillus salivarius*, *Veillonella parvula*, *Streptococcus salivarius*, and *Bifidobacterium*) in stool compared to controls and patients with alcohol use disorder without cirrhosis.[45] Furthermore, pro-inflammatory bacteria such as *Enterobacteriaceae* were increased in patients with alcohol dependence, whereas butyrate-producing species (*Clostridiales*) were decreased.[45] Specifically, cirrhosis was significantly associated with the presence of *Bifidobacterium*. The *B. dentium* species, linked to alcohol-associated cirrhosis, has been shown to play an important role in GABA production.[4]

Another study analyzed microbiota in the colons of healthy controls as well as 48 patients with AUD with and without liver disease.[46] Mutlu et al. suggested that dysbiosis was worse in patients with alcohol-associated cirrhosis than in those with cirrhosis from other causes. Their study demonstrated that even in the early stages of ALD (without cirrhosis), changes in the gut microbiome occurred, such as reduced *Bacteroidetes* and increased *Proteobacteria*, and that levels of endotoxin were higher in patients who consumed alcohol.[46] Alcohol also has been shown to decrease commensal taxa in patients consuming alcohol, irrespective of their cirrhosis status.[47] It is suspected that increases in oral microbiota in the stool of patients with cirrhosis could be a result of the higher rate of oral infections, changes in salivary microbiome, and use of acid-lowering medications in this population. One study also suggested that increasing severity of liver disease is associated with a relative decrease in *Akkermansia muciniphila*.[48] Therefore, changes to the gut microbiome may be influenced by the severity of liver disease.

Fungi

Studies in people with ALD have identified an increase in *Candida* species and a decrease in *Epicoccum*, *Galactomyces*, and *Debaryomyces*. Lower fungal diversity was observed in patients with ALD compared to healthy controls. In addition, these changes to the intestinal mycobiota were consistent among patients with varying degrees of ALD.[49,50]

Viruses

The link between viruses and ALD is complex, and current knowledge is limited.[51,52] In patients diagnosed with alcohol-associated hepatitis, phages with hosts as varied as *Escherichia*, *Enterobacteria*, and *Enterococcus* were increased, as were viruses such as Parvoviridae and Herpesviridae. Specifically, the severity of ALD was associated with the presence of *Staphylococcus* phages and Herpesviridae.[52]

Effects of gut microbiota modulation

Several studies have assessed the effects of modulation of the gut microbiota on ALD. In a double-blind, placebo-controlled study, Amadieu et al. assigned a prebiotic (inulin) versus placebo for 17 days to 50 patients with ALD.[53] Patients receiving inulin had significantly higher markers of hepatic inflammation. In the subset of patients who had early ALD (as defined based on FibroScan and serum values), inulin administration was linked to an increase in *Bifidobacterium* and a decrease in *Bacteroides*, and again, higher levels of hepatic inflammation. These findings suggest that inulin may be able to alter the gut microbiome but not necessarily lead to clinically apparent changes to inflammation and that prebiotics may not be successful or beneficial for improvement in liver parameters. This study was limited, however, by sample size and a relatively short duration of inulin administration. Another study assessed the effects of LGG use in patients with moderately severe alcohol-associated hepatitis. LGG was associated with reduced short-term liver injury and reduction of alcohol consumption to abstinence levels at 6 months.[54]

The role of SCFAs also has been explored in patients with ALD. A metabolomics analysis of fecal specimens demonstrated changes in tetradecane, reduced antioxidant fatty alcohols, and reduced SCFAs.[55] These alterations promote an environment prone to oxidative stress and increased gut permeability.

Role of FMT in AUD Treatment

Another area of interest has been the role of FMT in AUD treatment. Bajaj et al. demonstrated the safety of FMT in patients with alcohol-associated cirrhosis.[56] They concluded that FMT was associated with reduced alcohol consumption

and craving, with higher SCFA and microbial diversity. There was also a nonsignificant trend toward abstinence in the FMT group. Wolstenholme et al. further explored these mechanisms in a cross-species FMT design, mentioned below.[41] A larger trial studying the clinical efficacy of FMT (NCT05548452) is currently enrolling.[57]

To extend these findings, Philips et al. treated patients with severe alcohol-associated hepatitis with FMT and prospectively analyzed stool samples.[58] During a follow-up period of up to 3 years, patients who underwent FMT had lower rates of ascites, encephalopathy, infections, and hospitalizations with higher survival rates. Moreover, the FMT group demonstrated decreased alcohol relapse rates and longer time to relapse when compared to the standard-of-care group. Regarding microbiota composition, the FMT group demonstrated an increase in *Bifidobacterium* and a decrease in *Acinetobacter*, thus favoring a nonpathogenic milieu.

In patients with severe alcohol-associated hepatitis refractory to steroid therapy, liver transplantation, with the limitations described above, typically is the next treatment option. To address this, an open-label study was conducted with eight patients who were ineligible for steroid therapy and were treated with nasojejunal FMT for 1 week.[59] Patients treated with FMT were found to have higher transplant-free survival, associated with reduction in pathogenic bacteria, as compared to historical patients with steroid-refractory alcohol-associated hepatitis (87% vs. 33%). Specifically, at the 1-year follow-up, patients treated with FMT had fewer *Proteobacteria* and more *Actinobacteria*. Furthermore, they exhibited a relative increase in nonpathogenic bacteria such as *Enterococcus villorum* and *Bifidobacterium longum*. Notably, there was coexistence of recipient and donor species at 6 and 12 months after FMT.[59]

The benefit of steroid treatments for severe alcohol-associated hepatitis is modest and limited to 28-day survival. Patients with alcohol-associated hepatitis have microbiota changes characterized by predominance of pathogenic species leading to immune dysregulation. Another study comparing FMT in 13 patients with standard of care (without steroids) in 20 patients reported a statistically significant increase in 90-day survival with FMT (54% vs. 25%, $p = 0.02$).[60] In an extension of these two studies,[59,60] Pande et al. compared the safety and efficacy of healthy-donor FMT versus prednisolone therapy in patients with severe alcohol-associated hepatitis in an open-label study; each group included 60 patients.[61] There was a statistically significant improvement in 90-day survival in the FMT group compared to the prednisolone group (75% vs. 57%, p = .044). Moreover, there were significantly fewer deaths related to infections in the FMT group, suggesting that FMT can be a safe alternative in patients with severe alcohol-associated hepatitis. However, further studies are needed with differing formulations.

FMT and Gut-Brain Axis Changes in ALD: Clinical Studies

A randomized controlled trial of FMT enema of men with cirrhosis and recurrent hepatic encephalopathy found that FMT increased microbiota diversity and improved cognition compared with standard of care.[62] Using a rationally derived stool donor that was enriched in SCFA-producing *Lachnospiraceae* and *Ruminococcaceae*, this open-label randomized controlled trial with a follow-up period of 5 months found that with antibiotic pretreatment and administration of an FMT enema, the FMT was significantly better tolerated than the standard of care treatment.[62] Whereas five patients in the standard of care group developed hepatic encephalopathy, none of the patients who had received FMT did. Other benefits associated with FMT included improved cognitive performance and changes in the microbiome, such as relative reduction in nonpathogenic taxa and increased microbial diversity.[62] A subanalysis of the data showed that improvement in microbial function was linked to cognitive improvement.[63] Long-term follow-up of participants in this trial showed a continued relative increase in *Burkholderiaceae* and decrease in *Acidaminococcaceae* in the FMT group.[64] Furthermore, the FMT group had decreased rates of liver-related hospitalizations and hepatic encephalopathy recurrence, suggesting that FMT could significantly improve the clinical course of patients with cirrhosis and have a positive impact on quality of life as well as reduce the economic burden of hospitalization.[64]

The effect of orally administered FMT on the gut-brain axis in cirrhosis also was studied in a phase I, randomized, placebo-controlled trial. Cognitive function improved after FMT, as measured by performance using the EncephalApp.[65] The study also confirmed the primary endpoint of safety and tolerability of the oral FMT capsules.[65] FMT also improved mucosal diversity, dysbiosis, and microbial function.[66]

Cross-species studies of microbiota and AUD

In one of the first cross-species studies, the gut microbiota from patients with AUD increased alcohol preference, induced changes in anxiety-like and depression-like behaviors, and altered brain gene expression of recipient mice.[38] The fecal microbiome of men hospitalized for AUD (Alc-FMT), enriched in *Firmicutes* and *Bacteroidetes*, or of the control group of men who had abstained from alcohol for at least a year (control-FMT) was transplanted over 13 days into male mice that had been pretreated with antibiotics. Alcohol intake and preference for 4% or 8% alcohol in a two-bottle choice model were increased in the Alc-FMT mice compared to control-FMT mice. Alc-FMT mice also showed decreased anxiety-like behavior (indicated by increased time in the open arms of the elevated plus maze or in the center of an open field), increased depression-like behavior (indicated by immobility in the tail suspension test), and fewer

social interactions compared to control-FMT mice. With respect to gene expression, Alc-FMT mice showed reduced expression of the metabotropic glutamate receptor 1 (*mGluR1*) and *PKCε* mRNA in the nucleus accumbens and reduced *Bdnf* and $GABA_A$ receptor (alpha-1$GABA_AR$) expression in the medial prefrontal cortex. Of note, antibiotic treatment prior to FMT modified some behaviors (e.g., decreased anxiety-like behavior) and increased locomotor activity in some tasks; however, social interactions and depressive-like behavior were not altered. Overall, the findings demonstrated that the gut microbiome of heavy drinkers can transmit some behavioral phenotypes similar to those seen in human drinkers.[38]

A separate study extended these cross-species findings by investigating the effects of an alcohol-FMT on addiction-associated behaviors such as sociability, anxiety-like and depression-like behavior; on brain functions such as myelination, neurotransmission, and inflammation; and on intestinal bacterial load and permeability.[43] Mice that received an FMT from patients with AUD with severe symptoms of gut dysbiosis; high depression, anxiety, and alcohol craving; and low sociability also displayed deficits in a social preference task and higher depressive-like behavior; however, no differences were found in models of anxiety-like behavior.[43] This was accompanied by increased corticosterone levels compared to mice that received control FMT. Within the brains of Alc-FMT mice, expression of several neurotransmitter subunits and myelin-associated genes was altered, but pro-inflammatory cytokines, chemokines, and markers of microglial activation were increased in the striatum, but not the prefrontal cortex, suggesting a local inflammatory response. Total bacterial load in the intestine was reduced in Alc-FMT mice, suggesting a lower bacterial count. The relative abundance of *Bacteroidetes* was decreased, while the abundance of *Firmicutes* was increased, similar to what is found in patients with AUD. This was accompanied by indicators of increased intestinal permeability, including decreased expression of markers of defense immune mechanisms, loss of intestinal homeostasis (reduced expression of *Reg3g* and *Lcn2*), modification of tight junction expression, and atrophy of the mucosal structure (reduced villous height and crypt depth in the ileum). Interestingly, the study suggested that the behavioral changes may not have been induced through a peripheral inflammatory response, but rather may have been a result of blood metabolite changes. Although the FMT-treated mice were not exposed to alcohol, increased portal vein ethanol concentrations were found in Alc-FMT mice. This suggests that the Alc-FMT mice likely were colonized by higher amounts of alcohol-producing bacteria such as *Clostridium*, *Lactococcus*, *Turicibacter*, and *Akkermansia*.

In a third study using a cross-species FMT design, changes in alcohol preference and intake that occurred in patients with AUD after receiving a fecal transplant were transmissible by FMT to germ-free mice (i.e., which had been treated to lack any microorganisms).[41] The study used fecal samples from a randomized clinical trial that demonstrated reduced alcohol craving and consumption after fecal transplantation in patients with severe AUD. Germ-free male mice then received either stool or sterile supernatants (the nonmicrobial buffer collected from around the stool pellet) collected from trial participants pre-/post-fecal transplant. Mice colonized with postfecal transplant stool but not supernatants exhibited reduced alcohol acceptance, intake, and preference compared with mice receiving prefecal transplant stool. Analyses of gene expression in the liver, intestine, and prefrontal cortex revealed that a majority of the differentially expressed genes—which were related to immune response, inflammation, oxidative stress response, and epithelial cell proliferation—occurred in the intestine rather than in the liver or prefrontal cortex.[41] These findings suggest a potential for therapeutically targeting gut microbiota and the microbial-intestinal interface to alter gut-liver-brain axis and reduce alcohol consumption in humans.

Conclusions and Future Directions

The studies reviewed here demonstrate the role of the gut microbiome in AUD and ALD. They suggest that the use of probiotics, prebiotics, or FMT warrants further investigation as therapeutic approaches for these conditions. In clinical and preclinical studies, excessive drinking or exposure to high levels of alcohol was associated with dysbiosis, intestinal permeability, and changes in immune response (see Table 1). Clinical studies have suggested that use of FMT in patients with AUD improved SCFA levels, which may reduce inflammation and aid in preventing additional liver damage.[56] FMT also has recently been used in preclinical models to manipulate the gut liver axis with certain dietary supplements to alleviate signs of acute or chronic alcohol-induced liver disease.[32-34]

Preclinical studies have used probiotics, prebiotics, or FMT from animals that had consumed those substances to improve alcohol-related behaviors such as alcohol consumption, providing evidence that gut microbiome manipulation may improve not only inflammation-related markers, but alcohol-related behaviors as well.[41,42] Several of the preclinical studies identified in this narrative review were proof-of-concept FMT studies to show that behaviors such as anxiety-like and depression-like phenotypes and alcohol drinking can be induced by FMT from a donor with a history of alcohol exposure.[38-40,67] However, the body of evidence in regards to FMT studies currently is still limited.

Clinical data suggest that with strict donor screening protocols, FMT appears to be safe, with low incidence of

reported adverse events; however, long-term prospective data are still lacking.[56] Currently, FMT only is indicated for recurrent *Clostridium difficile* infection, but the mounting evidence from preclinical and clinical studies suggests that it may be a therapeutic option for ALD as well.[47] Several challenges exist, however, including the need to define a healthy stool donor, determine the optimal route of FMT administration, and find effective ways to validate endpoints. Changes in the microbiome can lead to progression of ALD by maintaining a state of localized and systemic inflammation.[46] Also, although human studies support the role of healthy-donor FMT in improving transplant-free survival, reducing rates of infections, and even ameliorating craving for alcohol in patients with AUD, clinical data are limited by small sample sizes. Moreover, these studies often have focused on advanced ALD, and the benefit of FMT intervention on the liver and on psychological parameters in patients with less advanced forms of ALD remains unknown.

A study that compared pentoxifylline, corticosteroid, and nutritional therapy with FMT found that patients who received FMT via nasojejunal route had the highest survival rates of all groups at 3-month follow-up, suggesting a possible mortality benefit for FMT. FMT also led to improvement in clinical parameters while modulating and targeting inflammatory pathways such as LPS.[68] Therefore, when compared to other medical interventions such as steroids that have side effects, FMT could potentially serve as a relatively benign treatment modality. However, a major limitation to this study was inclusion of only male patients, which raises the question of generalizability.

Understanding of the role of the microbiome in progression of ALD is growing rapidly. However, questions remain regarding its exact role in the pathophysiology of liver disease and in therapeutic strategies. Although abstinence remains the cornerstone of therapy for AUD, the point at which abstinence can modulate changes at the microbiome level is poorly understood. Future studies should focus on the composition and function of the microbiome and its byproducts at the various stages of the ALD spectrum. This will require large, prospective clinical trials with a diverse population sample. Although preclinical studies have suggested that manipulation of the gut microbiome may alter drinking behavior, few clinical trials of microbiome-targeted interventions have assessed drinking behavior as an endpoint. Such studies would be important in assessing the impacts of FMT on AUD outcomes outside of ALD. The gut-brain axis also is known to play a critical role in AUD, as demonstrated by individuals with AUD having increased gut permeability that leads to higher rates of depression, anxiety, and alcohol craving after a short period of abstinence.[12] These observations suggest the microbiota can modulate cravings and other psychiatric comorbidities associated with addictive behaviors.

Dysbiosis occurs in some patients across the spectrum of liver disease severity, and changes in the microbiome are evident at the bacterial, viral, and fungal community level. Probiotics may address these changes; however, although probiotics have been associated with improvements in direct and indirect markers of disease severity in patients with ALD, most studies only had a small sample size, had a heterogeneous trial design, and were rarely reproduced. Targeting bacterial metabolites also could be promising, and given that patients with ALD have reduced levels of total fecal bile acids and SCFAs, addressing these changes could be a potential therapeutic target.

In summary, this review highlights the fact that, to date, few studies have evaluated FMT as a therapeutic option for reducing symptoms associated with excessive alcohol use. However, the number of such investigations is growing, and early studies have shown remarkable potential with a good safety profile. Although additional, larger clinical studies still are needed to determine whether FMT is an effective therapeutic strategy, evidence to date suggests that targeting the gut microbiome could be a promising treatment option for decreasing the risk of relapse in AUD patients and ameliorating the severity of ALD.

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Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes: A Narrative Review and Methodological Considerations

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PURPOSE: Although abstinence is recommended in pregnancy, many pregnancies are exposed to alcohol. Observational studies of the effects of low to moderate prenatal alcohol exposure (PAE) and neurodevelopmental outcomes have yielded inconsistent results, with some studies finding an increased risk of adverse neurobehavioral and cognitive outcomes, and other studies finding no changes or reduced risk of the same outcomes. The purpose of this narrative review is to summarize these inconsistencies and apply a methodological framework to discuss how different parameters contribute to the findings. The authors also provide recommendations on how to advance future research in this area.
SEARCH METHODS: The PubMed, Web of Science, and Embase databases were searched, along with reference lists of selected systematic reviews and meta-analyses. Search terms used were (infant or child or children or adolescent or offspring) AND (low or light or mild or moderate or low-to-moderate) AND (drinking or alcohol or drinks) AND (pregnancy or prenatal or fetal) AND (neurodevelopment or behavioral or psychological or cognitive or developmental) NOT (mice or rat or fish or animal) NOT (meta-analysis or review). Peer-reviewed original research studies were included if they analyzed associations between an exposure defined and characterized as low/light or moderate PAE with offspring neurodevelopmental outcomes. Animal studies, studies that did not provide clear cutoff points to classify PAE categories, studies lacking an abstinence control group, and studies that did not present a multivariable-adjusted measure of association were excluded.
SEARCH RESULTS: The searches identified 2,422 papers, with 36 papers meeting eligibility criteria. These studies were carried out across nine countries and included samples ranging from approximately 500 to 40,000 participants. Cognitive, academic, socioemotional, and behavioral outcomes were assessed from infancy through age 19.
DISCUSSION AND CONCLUSION: When the findings from the selected articles were summarized by geographic region, exposure definition, or neurodevelopmental outcome, no consistent observations or patterns emerged between low to moderate PAE and offspring outcomes. Although some studies found positive (i.e., beneficial) associations between low to moderate PAE and outcomes (primarily outcomes related to cognition) and others found negative (i.e., detrimental) associations (primarily for behavioral outcomes), most findings were null (i.e., showed no effect of PAE). The heterogeneity in study results is likely due to methodological issues, including residual confounding, effect measure modification, and exposure misclassification that make synthesis of studies difficult. Alternative study designs, including longitudinal trajectory analysis, sibling design, negative controls, and instrumental variable analyses, may reduce biases and are discussed. To date, the consequences of light to moderate levels of PAE on neurodevelopment remain unresolved; studies that advance methodological rigor will be important contributions to the field.

KEYWORDS: alcohol; prenatal alcohol exposure; fetal alcohol spectrum disorders; epidemiology; low, light, or moderate exposure; neurodevelopment

Prenatal alcohol exposure (PAE) is a necessary cause of fetal alcohol spectrum disorders (FASD), a group of alcohol-related conditions characterized by neurodevelopmental problems. Although PAE is associated with many adverse physical, neurodevelopmental, and social outcomes, the most commonly studied are neurodevelopmental—primarily behavioral and cognitive—outcomes. Associations between heavy PAE (which is inconsistently defined) or binge PAE (defined as consuming four or more drinks in about 2 hours in women, or the amount of alcohol necessary to achieve a blood alcohol concentration of 0.08% or higher[1]) and adverse neurodevelopmental outcomes have been well documented in the literature.[2-4] However, findings regarding associations between lower levels of PAE and neurodevelopmental outcomes are inconsistent, with summations to date yielding, at best, inconclusive results.[5] Moreover, there is no consensus in the literature on the definition of "low to moderate" PAE—or, correspondingly, the level of harm that low to moderate PAE may cause[5]—leaving pregnant individuals and their clinicians ill-equipped to assess risk of exposure.

Several systematic reviews[6-10] and at least four meta-analyses[2,11-13] have assessed associations between low to moderate PAE and child neurodevelopmental outcomes. Pooling results from studies published through 2012, Flak and colleagues reported a small positive association between mild to moderate PAE (defined as up to six drinks per week) and child cognition (beta estimate 0.04; 95% confidence interval *CI* [0.00, 0.08]); seven studies).[2] They also identified a modest association between moderate PAE (defined as up to six drinks per week, including some individuals who consumed at least three drinks per week) and adverse behavioral outcomes, such as problems with behavior regulation and increased demand for attention at ages 9 months to 5 years (beta estimate -0.15; 95% *CI* [-0.28, -0.03]; three studies).[2] A more recent meta-analysis, pooling studies published through 2020, also found that low to moderate PAE (author characterized, or one to fewer than seven drinks per week) was associated with adverse behavioral outcomes (i.e., attention problems) at ages 6 to 17 (*OR* 1.21; 95% *CI* [0.88, 1.65]; six studies). However, the magnitude of associations estimated varied dramatically across studies.[11] Dissimilar to the prior studies, a meta-analysis specifically examining the effect of low to moderate PAE (≤ 20 g/week to ≤ 50 g/week) on risk of attention-deficit/hyperactivity disorder (ADHD) reported no effect (*OR* 0.96; 95% *CI* [0.86, 1.02]; six studies).[12] Studies included in this review used a few different measures to assess ADHD symptoms between ages 3 and 14.[12]

The impetus remains to better understand the relationship between low to moderate PAE and offspring neurodevelopmental outcome. Although most authoritative bodies recommend complete abstinence from alcohol during pregnancy, PAE continues to be common, particularly in the early weeks of gestation prior to pregnancy recognition. In surveys conducted by the Behavioral Risk Factor Surveillance System between 2018 and 2020 in the United States, about 14% of pregnant women reported past 30-day alcohol use.[14] It is possible that the inconclusiveness in previous research findings is not driven by a paucity of research, but by inconsistencies in methodology used across studies. The purpose of this narrative review is thus threefold. First, it briefly summarizes select literature of low to moderate PAE and neurodevelopmental outcomes, noting consistencies and inconsistencies in findings. Second, it reviews methodological issues that limit valid ascertainment of the effects of low to moderate PAE on offspring neurodevelopmental outcomes. Third, it discusses alternative study designs that may address key methodological issues for consideration in future research.

Methods

Search Strategy

The PubMed, Embase, and Web of Science databases were searched on June 13, 2022. The search terms used to identify articles were (infant or child or children or adolescent or offspring) AND (low or light or mild or moderate or low-to-moderate) AND (drinking or alcohol or drinks) AND (pregnancy or prenatal or fetal) AND (neurodevelopment or behavioral or psychological or cognitive or developmental) NOT (mice or rat or fish or animal) NOT (meta-analysis or review). Results of the search strategy were checked against reference lists of existing systematic reviews and meta-analyses to verify that the search was comprehensive.[2,6,11,12]

Eligibility Criteria

The inclusion criteria for this review were: (1) peer-reviewed original research study; (2) human participants; (3) PAE characterized as low, light, mild, moderate, or low to moderate; and (4) any neurobehavioral or developmental outcome in offspring. Exclusion criteria were: (1) reference group other than "no PAE" or abstinence; (2) no parameters provided for the quantity or frequency of alcohol exposure that was used to classify individuals as having low, light, mild, moderate, or low to moderate PAE; (3) inability to separate PAE from co-occurrence with exposure to other substances; (4) no adjustment for confounding variables; (5) no measure of association presented for low/light or mild/moderate PAE categories (e.g., exposure was analyzed as a continuous variable); (6) quasi-experimental study design; and (7) alcohol exposure not specific to the pregnancy period.

Data Abstraction and Synthesis of Results

One of two reviewers examined titles and abstracts of each article to identify articles meeting criteria for full text review. During full text review, one of two reviewers abstracted the following information from each study: (1) author and year;

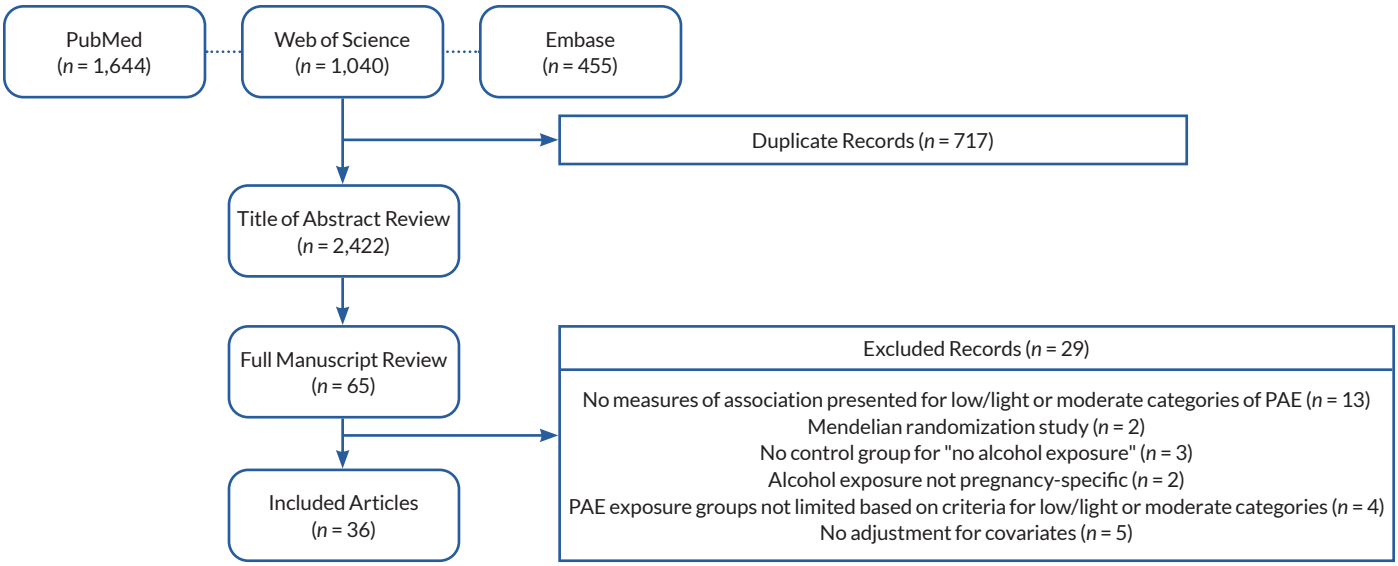

Figure 1. Sample selection for inclusion in this review. *Note:* PAE, prenatal alcohol exposure.

(2) data source and sample size; (3) study setting and offspring birth years; (4) definition of low/light PAE; (5) definition of mild/moderate PAE; (6) definition of one drink or one unit of alcohol; (7) timing of PAE measurement; (8) outcome, outcome measurement tool, and age of offspring at outcome measurement; (9) confounding variables considered; and (10) results. These data are summarized in Appendix 1.

Results

The search of PubMed (*n* = 1,644), Embase (*n* = 455), and Web of Science (*n* = 1,040) databases yielded 2,422 unique records, 65 of which met criteria for full text review. Of these, 29 were excluded (see Figure 1 for details of exclusions), and 36 were included in the final set. With a few exceptions, most studies reported no associations between low or moderate PAE and offspring neurodevelopment. Appendix 1 synthesizes the results by study location, exposure definition, timing of exposure measurement, and neurodevelopmental outcome.

Study Location

Included studies reported data from Australia (eight studies);[15-22] New Zealand, Australia, Ireland, and the United Kingdom (one study);[23] Denmark (nine studies);[24-32] Denmark and Finland (one study);[33] Japan (one study);[34] South Africa (one study);[35] the United Kingdom (nine studies);[36-43] and the United States (six studies).[44-49] Of note, many of the studies published from the same country used data from the same source. For example, half of the Australian studies used the Western Australian Health Survey data;[15,16,19,21] seven of the nine Danish studies used data from the Lifestyle During Pregnancy Study (based on a sample from the Danish National Birth Cohort);[26-32] and six of the nine U.K. studies used data from the Millennium Cohort Study.[36-39,41,50] Individual studies with the same data source often used the same definition of PAE but varied by study outcome or offspring age at neurodevelopmental assessment.

Some patterns were observed in study findings by country. For example, all studies from Denmark and Denmark/Finland reported mostly no effect of low to moderate PAE (i.e., null findings). Two studies—one using data from the Danish National Birth Cohort[25] and one using data from the Aarhus Birth Cohort[24]—published findings suggesting potential protective associations between low PAE and ADHD in both sexes[24] and between low to moderate PAE and internalizing problems among boys.[25] Results from each of the Australian studies were also mostly null, although two studies reported some evidence for worse behavioral problems with low PAE,[15,17] and three studies reported some evidence for better behavioral,[16] cognitive,[18] and academic outcomes with low PAE.[21] Studies from the United Kingdom focused on potential sex differences in the associations between low to moderate PAE and neurodevelopmental outcomes. Two studies using data from the Avon Longitudinal Study of Parents and Children reported worse behavioral rating scores among girls (but not boys) exposed to light PAE compared to children without PAE.[40] Two analyses using data from the Millennium Cohort Study reported that light PAE was associated with better cognitive scores[36,37] and lower behavioral scores[37] among boys relative to boys without PAE. Of the six studies from the United States, findings from three studies suggested worse behavioral outcomes with light to moderate PAE compared with no PAE,[45,46,48] one study noted a possible protective association

between light PAE and autism spectrum disorder (ASD),[44] and the remaining two studies showed null results[47,49] (see Appendix 1).

Prenatal Alcohol Exposure

There was tremendous heterogeneity in how low/light or moderate PAE were defined. Most studies included in this review defined these categories based on the average quantity of drinks consumed per week. Of these, low/light PAE was most frequently defined as averaging one to four units of alcohol per week, with specific definitions ranging from less than one unit per week to less than seven units per week. The studies using the higher threshold of fewer than seven units per week were mostly conducted in Australia, matching the definition of low-risk drinking for women.[15-17,20,21,52] Many studies also considered units per occasion in their drinking definition. For example, most studies using data from the Millennium Cohort study defined low PAE as "not more than one to two units per week or per occasion;"[36-38,50] and most studies using the Western Australian Health study defined low PAE as "not more than seven drinks per week and up to two drinks per occasion."[15,19,20,21,52] The study from South Africa considered only the number of drinks per occasion (mild to moderate: not more than three drinks in one sitting, never binge drinking),[35] and the study from Japan considered only frequency of drinking (low PAE: drinking rarely to one to four times per month).[34] For moderate PAE, most definitions were between three and 10 drinks per week. Some considered drinks per occasion in addition to drinks per week (range from more than two to five drinks per occasion).

The definition of a drink or unit of alcohol also varied by country. A standard drink was typically defined as 10 grams of pure alcohol in Australia; 12 grams in Denmark; and 8 grams or half a pint of beer, a glass of wine, or a single measure of spirits or liquor in the United Kingdom (see Appendix 1). No clear pattern of study findings emerged by definition of low/light PAE, moderate PAE, or unit of alcohol.

Timing of PAE Assessment

In all studies analyzed, PAE was assessed using self-report by the women as there are no biomarkers that could provide details on the dose and timing of low to moderate PAE. Twenty-two of the included studies collected self-report information on PAE during pregnancy,[16-18,20,22-33,35,40,42,43,47,49] and 14 studies relied on reports obtained after pregnancy,[15,19,21,34,36,37,39,41,44-46,48,50] including 10 studies with reporting occurring within 1 year of delivery.[15,19,21,36-39,41,45,50] Only three studies assessed PAE at the same time as or after the infant outcome was assessed (i.e., retrospectively). Of these, two reported that light to moderate PAE was associated with worse behavioral problems at age 23 months[48] and at ages 9 to 10 years;[46] and the third—a case control study examining associations with ASD—reported mostly null findings, with the exception of a protective association between one to two drinks in the first month of pregnancy and lower odds of ASD.[44]

Neurodevelopmental Outcomes

The studies included in this review assessed neurocognitive, academic, and behavioral outcomes when offspring were between the ages of 9 months and 19 years. None of the included studies reported worse neurocognitive outcomes with low to moderate PAE. For example, studies examining associations between PAE and Bayley Scales of Infant Development mental development and psychomotor indices in infants ages 12 to 24 months reported mostly null findings,[17,20,42,52] with one study suggesting that low PAE in the second and third trimesters was associated with better cognitive outcomes.[18] Three studies examining associations between PAE and IQ at about age 5 also reported null findings.[23,26,29] Four studies using data from the Millennium Cohort Study that assessed cognitive development with the British Abilities Scale reported protective associations between light PAE and cognitive outcomes in boys at age 3[36] and age 5,[37] but null associations when the boys were evaluated at ages 7 and 11.[38,39]

For measures of socioemotional and behavioral health, most studies used data ascertained with either the Strength and Difficulties Questionnaire (10 studies[25,30,33,36-41,43]) or the Child Behavior Checklist (CBCL; seven studies[15,16,22,23,34,35,46]). For these measures, offspring age at the time of assessment varied greatly and results were mixed with no clear pattern identified. Others studies examined PAE in relation to specific diagnoses, including ADHD (two studies)[24,41] and ASD (two studies),[44,50] with all studies reporting null or protective results. Three studies used infant behavior rating checklists to measure behavioral outcomes in infants ages 9 to 24 months. All three studies reported that light to moderate PAE was associated with worse behavioral outcomes in infancy, including increased infant difficultness,[48] poorer social engagement,[45] and more sensation seeking.[17]

Regarding academic outcomes, three studies reported null associations between PAE and academic achievement.[39,40,49] One study reported that low PAE was associated with lower odds of meeting Australian numeracy academic benchmarks, but had no effect on meeting reading, spelling, or writing benchmarks.[21]

Discussion and Comment

Despite a large and growing evidence base, making inferences about the effects of light to moderate PAE on neurodevelopmental outcomes in offspring remains difficult. Heterogeneity in effect estimates has persisted over the past 20 years, with both harmful and protective effects observed. Due to differences in study approach (summarized in Appendix 1) and methodological limitations, the diverse study results are difficult to synthesize. The following section further details these issues.

Methodological Issues With the Study of Light to Moderate PAE

Definition of exposure

Currently, there are no standard criteria or consensus for defining low/light or moderate levels of PAE. This heterogeneity in exposure definitions has undoubtedly contributed to inconsistencies of effect estimates and has limited the ability to make comparisons between studies. Even in abstracting data for this review, the authors were limited to papers that self-classified exposure into low/light or moderate PAE. Undoubtedly, many additional studies have investigated similar exposure levels but did not label them as such and therefore could not be included in this review because they were not identified in the literature searches.

Nondifferential exposure misclassification: Static measurement of PAE

Studies of PAE often use time-insensitive, or static, categorizations of exposure (e.g., categorizing the entire pregnancy as "high" consumption based only on PAE at conception) that fail to incorporate the dynamic changes in exposure that occur across pregnancy. These changes typically occur around the time of pregnancy recognition (which can be highly variable across individuals) when many women reduce their consumption or abstain from alcohol. For some pregnancies, changes also occur later in pregnancy as the perceived "risk period" for fetal development passes and women feel more comfortable resuming some level of alcohol use. The timing of these transition points may be informative with respect to offspring development but frequently is not examined. Further, when time-varying exposures are collapsed into static variables, a resulting exposure misclassification may lead to attenuation of effect estimates. This is particularly of concern when studying the consequences of low and moderate PAE, where modest effect estimates can disappear entirely due to exposure misclassification.

Differential exposure misclassification: Recall bias

There are no validated biomarkers that reflect prenatal exposure several years after birth; consequently, PAE is frequently measured by maternal recall. The gold standard approach in the collection of PAE information is through timeline follow-back[53] during pregnancy before the outcome is known, after which recall bias may occur. Due to stigma, individuals who perceive neurobehavioral problems in their offspring may be more likely to underreport their prenatal alcohol consumption relative to individuals who perceive no problems, which in cases of extreme underreporting could result in estimated protective effects of PAE. Although it is much more feasible to collect information retrospectively once children are at suitable ages for neurodevelopmental evaluation, the impact of this approach on internal validity must be critically evaluated.

However, when assessing studies that rely on recalled PAE, research has noted that recalled alcohol exposure is strongly predictive of pregnancy, dysmorphic, and neurodevelopmental outcomes.[54] Further, validation studies comparing prospective and retrospective reports found that retrospective reports of maternal drinking reflect higher levels of consumption than prospective reports obtained during the prenatal period.[55-58] Thus, although collection of consumption data during pregnancy remains preferable, retrospective information should not be discounted simply due to potential recall bias, and actually may be more accurate in some groups, particularly those who perceive stigma when reporting during pregnancy. Notably, however, the ability to recall PAE after pregnancy may differ by level of prenatal alcohol use. Women who did not consume any alcohol as well as those who habitually had high levels of consumption both may have more accurate recall than women who infrequently consumed alcohol, particularly with respect to the precise timing and amount of alcohol. This differential exposure misclassification could move estimates of PAE effects in either direction (toward or away from the null).[59]

Confounding variables

To validly estimate a causal effect of PAE on neurodevelopment, the exposure groups must be interchangeable. This means that variables that could confound the association between light to moderate PAE and offspring outcomes are equally balanced between alcohol-exposed and non–alcohol-exposed offspring. In observational studies, many socioeconomic and psychosocial factors have been associated with alcohol consumption patterns. A study of more than 6,000 women in Australia examined maternal factors associated with patterns of alcohol consumption before, during, and after pregnancy. The analysis found that compared to women with light prenatal alcohol consumption (0.4 drinks per day pre-pregnancy, early pregnancy cessation), women with high levels of alcohol consumption (2.5 drinks per day pre-pregnancy, 0.6 drinks per day during pregnancy) were more likely to have a lower income, be single or divorced, be pregnant for the first time, not attend church, report depression or anxiety, have high maternal adversity, and have adverse health-related lifestyle behaviors (e.g., smoking, little exercise, poor sleep).[60] Within the same study, women who abstained from alcohol pre-pregnancy through postpartum were also more likely to have lower income, have more children, and have adverse health-related lifestyle behaviors compared to women with light prenatal consumption.[60] Similarly, in a study of 4,000 pregnant women in the United Kingdom, women of higher socioeconomic status were more likely to drink wine, which was more likely to be consumed in low to moderate amounts, and less likely to binge drink than those with lower socioeconomic status. Further, being older, being better educated, having a higher social class, being employed, and having a better educated, employed partner were associated with consumption of wine, whereas smoking, lower education, and worse mental health

were more strongly associated with consumption of beer.[61] Other factors that favor positive neurobehavioral outcomes in the offspring were disproportionally shared by women who consumed low to moderate amounts of alcohol. These factors include better diets, earlier use of prenatal vitamins, lower prevalence of mental illnesses such as depression or anxiety, and lower likelihood to use other substances (e.g., marijuana) during pregnancy.[62] These studies highlight the imbalance in protective factors that often accompany low to moderate PAE that may bias findings, resulting in null or even protective effects when compared to abstinence.

Although these and other factors associated with low to moderate prenatal alcohol consumption that reduce the likelihood of adverse neurodevelopmental outcomes are routinely included in multivariable adjustment, they may still result in unmeasured confounding. Additionally, sample sizes typically limit the ability to include the multitude of variables necessary to establish true exchangeability. As a result (and common in all observational studies), the degree to which residual confounding persists—as evidenced by positive associations often reported between low to moderate PAE and offspring neurodevelopmental outcomes—must be considered because there is no conceivable benefit on these outcomes from PAE itself.[63,64]

Effect modification

In addition to confounding, which affects the internal validity (bias) of an estimate, the effect modification of the estimate by factors associated with the outcome (which can be termed modifiers or moderators) must be considered. Although not a source of bias, this occurs when the magnitude of the effect varies across levels of a third variable, which may contribute to heterogeneity in effect estimates across studies. Here, the authors hypothesize higher socioeconomic status associated with low to moderate PAE to be the third variable. For example, women consuming low to moderate levels of alcohol in pregnancy, which as previously noted is associated with higher socioeconomic status, may have more resources at their disposal postnatally compared to either women who abstain or women who consume large quantities of alcohol, providing a more enriched environment for the offspring. These factors include the likelihood to breastfeed, high-quality childcare, reduced environmental exposures, higher levels of social support, access to health care, reduced caregiver stress, and educational resources available to the child, which benefit neurodevelopmental outcomes. When effect estimates for PAE are not stratified by these potential modifiers, outcomes most vulnerable to PAE may be masked by the preponderance of protective factors associated with low to moderate PAE.

In summary, the null or protective effects attributed to low to moderate PAE on neurodevelopmental outcomes are likely, at least in part, attributable to unmeasured confounding associated with the exposure as well as to effect modification by postnatal factors that favor children with low to moderate PAE. Many statistical tools exist to address confounding (e.g., propensity score adjustment, inverse probability of treatment weights) and effect modification (e.g., stratification, re-weighting to a standardized sample). However, these options are imperfect because of the strong psychosocial patterning of PAE and insufficient resources to validly measure all potential confounding variables or examine associations across population subgroups. Further, as previously noted, there is a high degree of nuance in the operationalization of PAE, highly likely leading to misclassification of exposure and attenuation of results. To overcome these challenges, researchers have begun applying novel study designs and exposure models to this research. The following section highlights a few such approaches.

Alternate Study Designs to Address Methodological Issues

The study designs and methodologies discussed in this section—which are by no means exhaustive—specifically target some of the aforementioned threats to validity. Thus, exposure misclassification can be addressed by determining longitudinal trajectories of PAE, and confounding can be limited through use of instrumental variables, sibling designs, and negative control studies. These methods can be used alone or in combination to enhance estimation of causal effects.

Longitudinal trajectories of PAE

Efforts to better capture and operationalize the three parameters of alcohol use (timing, dose, and duration) have been ongoing for many years. Initially, researchers manually clustered individuals based upon these characteristics. In one study, investigators created a composite measure of PAE that incorporated dose, pattern, and timing of consumption into a descriptive, categorical variable (e.g., low, moderate, binge drinking less than once per week; binge drinking once or twice per week; high PAE).[15] When they compared outcomes using these classifications to traditional analytic methods (i.e., average quantity per trimester, average daily exposure across pregnancy, average weekly exposure across gestation), the researchers detected increased odds of anxiety or depression for children with moderate PAE in the composite models that were not evident in the traditional analyses.[15] Lately, unsupervised machine learning techniques have been incorporated into analyses to identify patterns of use across gestation.

Although several methodologies exist, the underlying premise of longitudinal exposure modeling is to create groups with similar longitudinal exposure patterns to minimize heterogeneity within the assigned trajectory group and maximize heterogeneity between trajectories. In a study employing PAE trajectories in Ukraine, sustained alcohol exposure, even at relatively low levels (about one drink per day), was associated with modest

reductions in neurodevelopmental performance at 6 and 12 months of age compared with trajectories of higher PAE with alcohol reduction or cessation earlier in pregnancy.[65] In the Safe Passage Study (South Africa and the United States), the sustaining trajectory (modeled as maximum drinks per drinking day) was associated with sudden infant death syndrome, whereas the trajectories with similar early pregnancy consumption but earlier cessation were not.[66] By modeling trajectories, researchers can disaggregate patterns of consumption, even among individuals with low to moderate levels of consumption, to better identify and understand the nuanced risk of adverse offspring outcomes that are often lost when exposure variables are operationalized as static measures.

Instrumental variables

Instrumental variable (IV) analysis is another study design that could improve research on low to moderate PAE and address issues of exchangeability. An IV affects the outcome only through its effect on the exposure and is unrelated to potential confounders of the exposure–outcome association.[67] In experimental studies, the treatment assignment is the IV. However, when experimental studies are not feasible, researchers may use a "quasi-experimental" approach to an IV through "Mendelian randomization," involving the use of genetic variants that influence the exposure but are unrelated to factors that confound the exposure-outcome relationship.[68] Three recent studies using data from the Avon Longitudinal Study of Parents and Children (ALSPAC) utilized this design.[61,62,68] In one study, investigators used analysis of a maternal genetic variant in the alcohol dehydrogenase gene *ADH1B* as an instrument for assessing PAE.[61] Individuals who carry the rare variant rs1229984 in *ADH1B* have greatly increased enzymatic activity in the oxidation of ethanol to acetaldehyde. This increased activity results in a faster reduction of blood alcohol levels and sharper rise of acetaldehyde in blood and organs, leading to symptoms such as increased heart rate and nausea. Individuals with this variant consume less alcohol, and correspondingly, the fetuses of mothers with the variant have lower PAE. These observations were born out in the ALSPAC data, making this a suitable IV candidate for low to moderate PAE.[61,62,68] When the researchers used a traditional analysis of the effects of any alcohol exposure, estimates were largely null, indicating no effect. In contrast, when they stratified the analysis by type of exposure (i.e., preferred type of beverage), they noted positive effects between wine consumption and offspring academic achievement, and negative effects between beer consumption and offspring academic achievement. Given that there should be no difference between the effects of beer and wine when both were converted into standard, equivalent doses, the researchers suggested that the different outcomes were due to the strong social gradient associated with choice of beverage. *ADH1B*, however, was unrelated to potential confounders, but was predictive of alcohol use in pregnancy and, by extension, alcohol exposure to the developing fetus. When authors conducted the IV analysis with *ADH1B*, they found negative effects between PAE and academic achievement at all ages analyzed (i.e., ages 7 to 16).[61] Similar results were obtained when repeating this analysis with cognitive and educational performance at age 8.[68]

The third study that utilized an IV analysis constructed the IV from the child's genotype (variants of the alcohol dehydrogenase enzyme). It was hypothesized that offspring alleles, which result in "fast" metabolism of ethanol, would protect against abnormal brain development in infants.[62] Among offspring born to women with moderate alcohol consumption (one to six units per week during pregnancy), negative associations existed between the presence of genetic variants associated with slow alcohol metabolism and IQ at age 8. These associations were not seen in children with no PAE.[62] Assuming these findings can be replicated, they are a promising avenue to control for confounding factors while estimating the effects of low to moderate PAE on child health. Researchers should continue to explore additional IVs, specifically searching for stronger IVs than the alcohol metabolism variants, which can be exploited in this framework.

Sibling controls

A third promising approach that also addresses issues of exchangeability is a sibling design. In their simplest form, sibling designs compare outcomes among exposure-discordant siblings, which accounts for many shared environmental and familial confounders that are exceedingly difficult to fully adjust for through multivariable analysis. Sibling studies often result in attenuated effect estimates between prenatal exposures and offspring neurodevelopmental outcomes compared to traditional study designs,[69] highlighting the challenges of residual confounding.

At least three studies have utilized sibling designs to control for shared genetic and environmental confounders when studying PAE.[34,70,71] In the first sibling study conducted on approximately 4,000 mother-sibling triads, researchers found that employing the sibling design attenuated the initial multivariable-adjusted results for PAE and attention/impulsivity problems in offspring. However, an association still existed between heavy PAE (≥ 5 days/week) and offspring conduct problems at ages 4 to 11.[70] In a second study, conducted with 15,000 mother-sibling triads in Norway, researchers detected no effects of low levels of PAE on offspring behavioral problems, and attenuated yet modest effects of "hazardous" PAE on behavioral problems at age 3 when accounting for siblings.[71] A third study, which included 1,600 sibling pairs in Japan, found that low PAE (drinking one to four times per month or rarely) was associated with greater anxiety problems and internalizing problems. In that study, effect estimates were magnified in the siblings analysis compared to initial multivariable-adjusted

results.[34] Although researchers must consider exposure discordance and factors that changed between births (e.g., birth order, maternal age, socioeconomic factors) that may bias results, this model is a promising strategy to mitigate the persistent problem of residual confounding.

Negative controls

Finally, another option that does not require identifying an IV or observing siblings is to employ a negative control design. Such designs compare the effects of PAE on offspring outcome with the effects of similar exposure with no biological relevance to the offspring (e.g., maternal exposure prior to conception or postnatally or similar levels of exposure of other individuals [partner exposure]).[67] Negative control designs alert the analyst to uncontrolled confounding because if any of the effect estimates among the negative controls are positive, the effect measure of interest is likely confounded. Putting this design to practice, researchers using paternal exposure during pregnancy as a negative control in the Avon Longitudinal Study of Parents and Children found no evidence that maternal alcohol and tobacco consumption during pregnancy were more strongly associated with childhood IQ than paternal alcohol and tobacco consumption.[72] In a second negative control study using ALSPAC data, researchers found that offspring of mothers who consumed any alcohol at 18 weeks of gestation had a 17% increased risk of having a diagnosis of depression at age 18.[73] There was no clear evidence of association between partners' alcohol consumption at 18 weeks of gestation and increased risk of offspring depression. The investigators concluded that the negative control comparison of paternal drinking provided some evidence that the association between PAE and depression at age 18 may be causal and warranted further investigation and replication.[73] Although one must critically evaluate the potential for causality by the selected negative control (e.g., epigenetic effects in the case of paternal exposure), if it is deemed that there is no plausible mechanism by which the negative control could affect the outcome, the types of analyses should be conducted and reported.

Conclusions

Limitations of This Review

When reviewing the findings presented here, several limitations should be considered. First, restricting findings to published research introduces the potential for publication bias. However, given that many studies reviewed here yielded null findings, it is unlikely that publication bias greatly affects the results. Second, the high degree of heterogeneity in methods and the exposure definition prevented a meta-analysis.

Third, and most importantly, although three databases were searched, this review is a narrative review only and should not be interpreted as a systematic review. The search process used search terms "low, light, mild, or moderate," and the authors then looked for predefined criteria for the categorization. Accordingly, there could be additional studies that included similar exposure ranges but did not use those terms; other studies may have included a population with a mean PAE that fell within a range described as low or moderate, but the study did not actually set minimum or maximum parameters for defining the exposure. Either scenario would have resulted in exclusion of those studies from this review. For example, Parry et al. examined several categories of alcohol exposure (i.e., 0 g/week, > 0–29 g/week, 30–59 g/week, 60–89 g/week, 90–119 g/week, and > 120 g/week)[74] that overlap with definitions of low/light or moderate PAE in many of the studies included here. However, the study was excluded because the authors did not label these categories as low, moderate, or high exposure. Similarly, Beauchamp et al. enrolled a birth cohort with a PAE group that had a median of 0.84 ounces absolute alcohol per day during the periconceptual period and 0.3 ounces absolute alcohol per day during pregnancy.[75] Although this sample may have overlapped with other samples in this review of moderate PAE, it likely also included infants who would have been categorized as low PAE and some who would have been categorized as high PAE, given that no minimum or maximum criteria were set for inclusion. Therefore, the study was excluded from this review. Attempting to include all such studies would require (1) searching for any study that had any measure of PAE, and (2) having clearly defined and accepted standards for categorizing low and moderate PAE. Such manual abstraction and classification in the absence of consensus definitions were beyond the feasibility of this review.

Fourth, the studies reviewed here assessed a variety of neurodevelopmental outcomes that may differ in sensitivity to alcohol based on the measure or the timing of administration. It was beyond the scope of this review to comment on each measure's sensitivity and psychometric properties, although both also may contribute to the heterogeneity in findings. Finally, this review was limited to neurodevelopmental outcomes. There are other health and behavioral outcomes potentially affected by PAE that manifest across the life course and also warrant systematic investigation.

Public Health and Clinical Implications

Although pregnant women may want to know whether there is a safe drinking threshold in pregnancy, this question is difficult to answer in human research. As seen in this review, findings are heterogeneous across studies, and many methodological limitations impair ability to validly estimate the potential consequences of low to moderate PAE. Moreover, the public should not confuse inconsistent evidence and insignificant findings to indicate absence of an effect. Accordingly, the only way to be certain to avoid adverse outcomes associated with alcohol exposure is to follow current guidelines to abstain from alcohol during pregnancy. Given that many women do not

plan pregnancy, those who have had alcohol exposure prior to learning they are pregnant should avoid continued use.

Summary and Future Directions

Although this review generally found null associations between low to moderate PAE and adverse neurodevelopment, the issue is far from resolved. There is no consensus in the literature on the level of harm that low to moderate prenatal alcohol exposure may cause, and the differential vulnerability resulting from influences such as concurrent exposures to other substances, genetics, and other factors may prevent a clear conclusion. Although the evidence base continues to expand, substantial methodological limitations impair synthesis of study findings. Use of alternative study designs may help advance research of the effects of low to moderate PAE on adverse outcomes, and the authors look forward to the expansion of these methodologies in the field. In addition, expanding reviews to capture other outcomes, including physical and behavioral outcomes, as they emerge across the life course is of great interest.

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Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Barbuscia et al. (2019)[39]	Millennium Cohort Study (*n* = 10,454)	United Kingdom 2000-2002	< 1-2 units per week per occasion	< 3-6 units per week or < 3-5 units per occasion	Half-pint of beer, glass of wine, or single measure of spirits or liquor	Interview at 9 months postpartum	British Ability Scale (BAS); ages 3, 7, and 11 Bracken School Readiness Assessment; age 5 Strengths and Difficulties Questionnaire (SDQ); ages 3, 5, 7, 11, and 14	Child gender, mother's ethnic background, low birth weight, maternal health, sociodemographic characteristics, and maternal cognitive ability	Light PAE was not associated with cognitive ability, internalizing, or externalizing behavioral problems. Moderate PAE was associated with better cognitive ability on 1 of 9 measures (others null) and worse externalizing problems at age 11 (null at other ages).
Bay et al. (2012)[27]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 685)	Denmark 1997-2003	Low to moderate: weekly alcohol intake 1 to 14 drinks per week Analyzed as categories: 1 to 4 drinks per week 5 to 8 drinks per week 9 to 14 drinks per week		12 g pure alcohol	Self-reported at 17 weeks of gestation	Movement Assessment Battery for Children; age 5	Paternal education, maternal IQ, prenatal maternal smoking, maternal age, parity, maternal binge drinking episodes during pregnancy, prenatal and postnatal marital status, postnatal parental smoking, maternal pre-pregnancy body mass index (BMI), child sex, age at testing, health status, hearing and vision on day of testing, family/home environment, physical activity	No association existed between low to moderate PAE and total motor impairment or any motor skill subscales.
Chen (2012)[48]	National Longitudinal Survey of Youth (*n* = 1,618)	United States 1986-2000	Light to moderate: < 3 or 4 days a month		Not reported	Maternal report postpartum; < 23 months	Modified Rothbart Infant Behavior Questionnaire; ages < 23 months	Sibling fixed effects model with control for prenatal smoking, poverty status, marital status during pregnancy, prenatal care in first trimester (T1), parity, child sex	Light to moderate PAE was associated with greater difficultness of infant. No associations were found with positive mood of fearfulness subscales.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Cluver et al. (2019)[35]	Safe Passage Study (*n* = 500)	South Africa 2007-2015	Mild to moderate: ≤ 3 drinks in one sitting; never binge		14 g ethanol	Modified alcohol timeline follow-back around conception, up to 4 times during pregnancy, and at 1 month postpartum	Kaufman Assessment Battery for Children (KABC-II), a developmental neuropsychological assessment (NEPSY-II), Preschool Child Behavior Checklist (CBCL); age 4	Maternal age, education, smoking, marijuana use, and methamphetamine use	No associations existed with neurodevelopmental or behavioral outcomes.
Falgreen Eriksen et al. (2012)[29]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 1,628)	Denmark 1997-2003	1 to 4 drinks per week	5 to 8 drinks per week	12 g pure alcohol	Self-reported at 17 weeks of gestation	Wechsler Preschool and Primary Scale of Intelligence—Revised; age 5	Parental education, maternal IQ, maternal smoking in pregnancy, child's age at testing, child's gender, parity, maternal marital status, maternal age and BMI, maternal binge drinking in pregnancy, family/home environment, prenatal/postnatal smoking, child's health status, hearing and vision abilities	No association existed between low or moderate PAE and IQ.
Forrest et al. (1991)[42]	Dundee antenatal clinics (*n* = 592)	Scotland 1985-1986	Mild: 1-49 g per week	Moderate: 50-99 g per week	10 g absolute alcohol (AA) (equivalent to one glass wine, half-pint beer, or one standard measure spirits)	Interviewer-administered questionnaire; T1, second trimester (T2)	Bayley Scales of Infant Development (BSID) mental development index (MDI) and psychomotor development index (PDI); 18 months	Maternal cigarette consumption, age, and social class; child's sex, birth weight, and gestational age	Mild PAE in early pregnancy was not associated with MDI or PDI. Moderate PAE in early pregnancy was not associated with MDI or PDI.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Gallagher et al. (2018)[50]	Millennium Cohort Study (*n* = 12,595)	United Kingdom 2000-2002	≤ 1-2 units per week or at any one time in pregnancy	≤ 3-6 units per week or 3-5 units at any one time in pregnancy	Half-pint beer, glass wine, or single measure spirits or liquor	Interview at 9 months postpartum	Maternal report of autism spectrum disorder (ASD) diagnosis; age 11	Parental age, household income, maternal education, social deprivation, ethnicity, marital status, maternal smoking, BMI, hypertension, diabetes, depression treatment, maternal smoking	No association existed between light or moderate PAE and ASD.
Goldschmidt et al. (2004)[49]	Maternal Health Practices and Child Development Study (*n* = 608)	United States 1984-1987	Light/moderate: < 1 drink per day		Not reported	Maternal interview at gestation months 4 and 7, and postnatally	Wide Range Achievement Test–Revised and Peabody Individual Achievement Test–Revised; age 10	Sociodemographic status, child characteristics and environment, maternal psychosocial status, current maternal substance use, and prenatal tobacco and illicit drugs other than marijuana	No association existed between light to moderate PAE and academic performance.
Halliday et al. (2017)[17]	Asking Questions About Alcohol in Pregnancy (*n* = 554)	Australia 2011-2012	≤ 20 g AA per occasion and ≤ 70 g AA per week	21-49 g AA per occasion and ≤ 70 g AA per week	10 g alcohol	Self-reported questionnaire at 13 weeks, 26 weeks, and postnatal interview; analyzed as T1, T2, and third trimester (T3)	BSID, Brief Infant-Toddler Social and Emotional Assessment; Infant/ Toddler Sensory Profile; age 2	Maternal age, maternal education, household income, ethnicity, language spoken in home, pre-pregnancy BMI, folic acid supplementation, smoking in pregnancy, age started drinking regularly, paternal drinking	Low to moderate PAE was not associated with most neurodevelopmental outcomes. Low PAE in T1 with abstinence in T2 and T3 was associated with more sensation seeking.
Hutchinson et al. (2019)[20,52]	Triple B Pregnancy Cohort Study (*n* = 1,324)	Australia 2009-2013	≤ 7 drinks per week and ≤ 2 drinks per occasion	≤ 7 drinks per week and > 2 to ≤ 4 drinks per occasion	10 g alcohol	Interview during T1, T2, and at 8 weeks postpartum; analyzed as T1, T2, and T3	BSID-III gross motor development, age 12 months	Age at birth, education, Socio-Economic Indexes for Areas, state of residence, country of birth, single-parent household, Aboriginal and Torres Strait Islander status, native language, substance use, pregnancy anxiety, IQ, parity, BMI, gestational age	No association existed between low or moderate PAE and gross motor development.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Ichikawa et al. (2018)[34]	Japanese Study of Stratification, Health, Income and Neighborhood (*n* = 1,600)	Japan 2010-2013	Drinking rarely to 1 to 4 times per month	Not applicable (N/A)	Not reported	Self-reported at offspring age 2-18 (same age as outcome measurement)	CBCL; mean age 9 (*SD* 4.4, age range 2-18)	Sibling analysis and adjustment for child's age, sex, parent's age, education, working status, family income, prenatal smoking, domestic violence, parental drinking	Low PAE was associated with greater anxiety problems, internalizing problems, and overall problems. Low PAE was not associated with several other CBCL-measured outcomes (e.g., externalizing behavior).
Kelly et al. (2009)[36]	Millennium Cohort Study (*n* = 9,460)	United Kingdom 2000-2002	≤ 1-2 units per week or per occasion	≤ 3-6 units per week or 3-5 units per occasion	Half a pint of beer, a glass of wine, or a single measure of spirits or liquor	Interview at 9 months postpartum	SDQ, BAS; age 3	Child's age, birth weight, mother's age at the time of birth, number of children in the household, mother's education, mother's drinking, mother's smoking habits, household income, pregnancy planned, mother's occupational class, mother's Kessler Psychological Distress Scale (K6) score, warmth of relationship between mother and child, parental discipline	Light PAE was not associated with behavioral or cognitive problems. In boys, light drinking was associated with lower conduct and hyperactivity problems, and higher cognitive ability. Moderate drinking was not associated with any outcome in either girls or boys.
Kelly et al. (2012)[37]	Millennium Cohort Study (*n* = 11,513)	United Kingdom 2000-2002	≤ 1-2 units per week or per occasion	≤ 3-6 units per week or 3-5 units per occasion	Half-pint of beer, glass of wine, or single measure of spirits or liquor	Interview at 9 months postpartum	SDQ, BAS; age 5	Child's age, birth weight, mother's age at time of birth, number of children in the household, mother smoked during pregnancy, pregnancy planned, parental income, highest parental educational qualification, highest parental occupation, mother's K6 score, parental discipline, child made to follow instructions, mother's parental competence, closeness of relationship between mother and child, mother's current drinking	No associations existed between light or moderate PAE and behavioral problems in girls or boys. Light PAE was associated with better cognitive ability scores in boys, null in girls; moderate PAE was not associated with cognitive ability scores.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Kelly et al. (2013)[38]	Millennium Cohort Study (*n* = 10,285)	United Kingdom 2000-2002	1-2 units per week or per occasion during pregnancy	N/A	Half-pint beer, glass of wine, or single measure of spirits or liquor	Interview at 9 months postpartum	SDQ, BAS; age 7	Mother's age, planned pregnancy, maternal smoking, parity, ethnicity, lone-parent family, life satisfaction, relationship quality, social networks, number of children in household, child's age, highest parental educational qualification, parental income, mother's mental health, parental discipline strategies, mother's self-rated competence, mother's closeness with child, mother's current drinking	No association existed between light PAE in pregnancy and behavioral or cognitive development.
Kesmodel et al. (2012)[26]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 1,628)	Denmark 1997-2003	1 to 4 drinks per week	5 to 8 drinks per week	12 g pure alcohol	Interview at 17 weeks	Behavior Rating Inventory of Executive Function, Wechsler Preschool and Primary Scale of Intelligence—Revised, Test of Everyday Attention for Children at Five; age 5	Parental education, maternal IQ, prenatal maternal smoking, child's gender, child's age at testing, test administrator, parity, maternal marital status, maternal age, maternal BMI, prenatal maternal average number of drinks per week, home environment, postnatal parental smoking, health status, hearing and vision abilities	No association existed between low or moderate PAE and executive function, intelligence, or attention.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Kilburn et al. (2015)[32]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 1,333)	Denmark 1997-2003	1-4 drinks per week	5-8 drinks per week	12 g	Interview at 17 weeks	Sternberg paradigm to assess information processing time and choice reaction time; age 5	Parity, prenatal maternal smoking, maternal pre-pregnancy BMI, length of parental education, marital status, postnatal parental smoking, child health status, family/ home environment index, breakfast irregularity, maternal depression, parental alcohol use, hearing ability, and vision ability	No association existed between maternal drinking and choice reaction time or information processing time.
Larkby et al. (2011)[47]	Clinical sample at maternity clinic (*n* = 592)	United States 1989-1991	Average drinks per day in T1 ≤ 0.4	Average drinks per day T1 > 0.4 to < 0.89	Not reported	Maternal report at 4th and 7th gestational months and at delivery	Computerized Diagnostic Interview Schedule-IV to measure conduct disorder; age 16	Prenatal exposure to tobacco, marijuana, cocaine, and other illicit drugs; income; race; gender; parenting style; life events; home environment; family history of alcohol problems; and maternal lifetime psychopathology	No association existed between light or moderate PAE and conduct disorder in adolescents.
Lees et al. (2020)[46]	Adolescent Brain Cognitive Development Study (*n* = 9,719)	United States 2005-2008	2.3 drinks per week (1st 7 weeks of pregnancy) 1.1 drinks per week through gestation	N/A	Not reported	Maternal report at offspring age 9-10 (same time as outcome measurement)	(1) CBCL, (2) Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS), (3) Impulsive Behavior Scale for Children–Short Form, (4) Behavioral Avoidance and Behavioral Inhibition Scales, (5) Cash Choice Task, (6) Rey Auditory Verbal Learning Test, (7) NIH Toolbox fluid intelligence battery, (8) brain imaging; ages 9-10	Birth weight, preterm birth, sex at birth, race/ ethnicity, youth age at time of assessment and school grade performance, maternal age at birth, maternal depression, and other substance use during pregnancy (tobacco, cannabis, cocaine)	Light stable PAE and light PAE reduced in pregnancy were associated with greater behavioral and psychological problems (CBCL: internalizing, externalizing, attention problems, total; K-SADS: anxiety, specific phobias) and differences in cerebral and regional brain volume.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Maher et al. (2022)[23]	SCOPE (Screening of Pregnancy Endpoints) and BASELINE (Babies After SCOPE: Evaluating Impact on Neurological and Nutritional Endpoints) (*n* = 1,507)	New Zealand, Australia, Ireland, United Kingdom 2004-2011	1-7 units per week		8 g or 10 ml (1 dl) pure alcohol	Interview at 15 weeks of gestation	CBCL; ages 2 and 5 Kaufman Brief Intelligence Test; age 5	Maternal age, maternal education, marital status, family income, maternal BMI, maternal smoking status at 15 weeks of gestation, and infant sex	No associations existed between low PAE and behavioral outcomes at age 2 or age 5.
McCormack et al. (2018)[18]	The Triple B Study (*n* = 1,331)	Australia 2008-2013	≤ 7 drinks per week and ≤ 2 drinks per occasion	≤ 7 drinks per week and > 2 to ≤ 4 drinks per occasion	10 g alcohol	Interview at T1, T2, and 9 weeks postpartum	BSID; age 12 months	Household SES, maternal age, maternal education level, Aboriginal or Torres Strait Islander origin, country of birth, single-parent household, first language, tobacco use, illicit substance use, anxiety, IQ, parity, and BMI	Low levels of PAE in T2 and T3 were associated with slightly higher cognitive scores. No associations existed with low or moderate PAE in T1. Moderate PAE in T2 and T3 was not analyzed.
Mitchell et al. (2020)[41]	Millennium Cohort Study (*n* = 13,004)	United Kingdom 2000-2002	≤ 3 to 7 drinks per week	≤ 8 to 14 drinks per week	Half a pint of beer, a glass of wine, or a single measure of spirits or liquor	Interview at 9 months postpartum	Parental report of attention-deficit/ hyperactivity disorder (ADHD) diagnosis, SDQ; age 7	Gender, gestational age at delivery, parity, paternal age, maternal age, maternal smoking status, maternal pre-pregnancy BMI, household income, maternal education, ethnicity, and marital status	Neither light nor moderate drinking were associated with ADHD, abnormal SDQ scores, or hyperactivity scores.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Niclasen et al. (2014)[25]	Danish National Birth Cohort (*n* = 37,152)	Denmark 1996-2002	Cumulative > 0 to 5 drinks per pregnancy; average > 0 to 2 drinks per week	Cumulative > 5 to 90 drinks per pregnancy; average 2 to 4 drinks per week	12 g pure alcohol	Maternal report at gestational week 16, week 30, and at 6 months postpartum	SDQ; age 7	Paternal smoking, parental education, parental pre-pregnancy psychiatric diagnoses, and maternal psychological well-being in pregnancy	No associations existed between light or moderate PAE with parent-rated conduct, emotional, hyperactivity/ inattention, or peer problems. Small protective associations existed between low and moderate PAE in early part of pregnancy with internalizing problems among boys (not girls).
O'Callaghan et al. (2007)[22]	Mater-University of Queensland Study of Pregnancy (*n* = 5,139)	Australia 1981-1984	Low: < 0.5 glass per day 0.5 to 1 glass per day		0.5 oz AA	Reported at first prenatal clinic visit and after delivery	CBCL; age 14	Cigarette smoking in early and late pregnancy, maternal BMI < 18.5, social risk score (low maternal education, maternal age < 19, single-parent status, or low income in pregnancy or at age 14)	No associations existed between light or moderate alcohol, neither early nor late in gestation, and attention, learning, or cognitive outcomes.
O'Leary et al. (2009)[19]	Western Australian Survey of Health (*n* = 1,739)	Australia 1995-1996	≤ 20 g alcohol per occasion, with a frequency of less than weekly up to 6 days per week	10 g to < 50 g alcohol per occasion, with a frequency ranging from less than weekly up to daily	10 g alcohol	Questionnaire at 12 weeks postpartum	Ages & Stages Questionnaire Communication Scale; age 2	McMaster's family functioning, parenting scale, partner present, maternal depression, anxiety, stress, maternal age at delivery, income, marital status, parity, education, smoking, use of tranquilizers, illicit drug use	No association existed between low PAE and language delay.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
O'Leary et al. (2010)[15]	Western Australian Survey of Health (*n* = 2,224)	Australia 1995-1996	< 70 g alcohol per week and ≤ 10-20 g per occasion	≤ 70 g alcohol per week and between 21 g and 49 g per occasion	10 g alcohol	Questionnaire at 12 weeks postpartum	Ages & Stages Questionnaire (language delay); age 2 CBCL; ages 2, 5, and 8	Antenatal covariates (maternal age, marital status, parity, ethnicity, income, maternal smoking, and use of illicit drugs, tranquilizers, and sleeping tablets during pregnancy), postnatal covariates (marital status, income, treatment for postnatal depression, postnatal depression, family functioning, parenting style, tension in the family due to alcohol and maternal depression, anxiety, and stress	No association existed between low PAE and language delay (also reported in paper above). Moderate PAE in T1 was associated with increased odds of anxiety/depression but not with somatic or aggressive problems. No association existed between low PAE and any CBCL outcome in children ages 2, 5, or 8.
O'Leary et al. (2013)[21]	Western Australian Survey of Health (*n* = 4,056)	Australia 1995-1996	1-2 drinks per occasion and < 7 drinks per week	3-4 standard drinks per occasion and ≤ 7 drinks per week	10 g alcohol	Questionnaire at 12 weeks postpartum	Western Australian Literacy Numeracy Assessment measures whether children met benchmarks for reading, writing, spelling, and numeracy; ages 8-9	Maternal age, education, marital status, ethnicity, parity, illicit and/or tranquilizer drug use, smoking, income, and languages spoken at home	Low PAE was associated with lower odds of missing numeracy academic benchmark; not statistically significant for reading, spelling, or writing benchmark. Moderate PAE was not associated with academic underachievement.
Robinson et al. (2010)[16]	Western Australian Pregnancy Cohort (*n* = 1,744)	Australia 1989-1991	Occasional: ≤ 1 drink per week Light: 2 to 6 drinks per week	7 to 10 drinks per week	10 g alcohol	Questionnaire at 18 and 34 weeks of gestation	CBCL; age 14	Maternal age, maternal education, presence of biological father in family home, family income, stress in pregnancy, maternal cigarette smoking, child's age	Light and moderate PAE in first 3 months of pregnancy was associated with lower internalizing and externalizing problem scores; no associations were observed for late pregnancy PAE.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Rodriguez et al. (2009)[33]	Three cohorts from Nordic Network on ADHD (*n* = 21,678)	Denmark, Finland 1984-2002	1 to 4 drinks per week	N/A	Not reported	Maternal report of average weekly exposure at ~16-32 weeks of gestation	SDQ, Rutter Scale; ages 7-15	Smoking, social adversity, birth weight, gestational age (analyzed in three cohorts to analyze differences in participant characteristics)	Low PAE was not associated with inattention/ hyperactivity.
Sayal et al. (2007)[43]	Avon Longitudinal Study of Parents and Children (*n* = 9,086)	United Kingdom 1991-1992	< 1 glass per week	N/A	8 g alcohol (equivalent to 1 glass)	Self-reported questionnaire at 18 weeks of gestation	Parental SDQ; 47 and 81 months Teacher SDQ; 92 to 108 months	Smoking, cannabis use, and use of illicit drugs in T1, highest level of maternal education, home ownership, marital status, parity, maternal age group, high Edinburgh Postnatal Depression Scale score, child ethnicity, gestational age group, and birth weight	Low PAE was associated with worse parental and teacher-rated SDQ scores among girls, but not among boys.
Sayal et al. (2013)[40]	Avon Longitudinal Study of Parents and Children (*n* = 10,558)	United Kingdom 1991-1992	< 1 glass per week	N/A	8 g alcohol (equivalent to 1 glass)	Self-reported questionnaire at 18 weeks of gestation	SDQ, Kay Stage 2 school examinations; age 11	Maternal age, parity, highest level of maternal education, daily frequency of smoking, use of cannabis and/or other illicit drugs during T1, home ownership, whether currently married, maternal mental health, child's gestational age, birth weight, and gender	No association existed between light PAE and outcomes. In girls, a suggestion of slightly worse outcomes appeared on parent-rated total SDQ score in those exposed to light PAE. Light PAE was not associated with Kay Stage 2 scores.
Singer et al. (2017)[44]	Study to Explore Early Development Case-Control Study (*n* = 2,515)	United States 2003-2006	Light: < 1 drink per week; 1-2 drinks per week		1 beer, 1 glass wine, 1 mixed drink, or 1 shot liquor	Maternal report* at 55 months postpartum (range: 29-68 months) *same time as outcome measurement	ASD diagnosis measured with the Autism Diagnostic Observation Schedule (child report) and the Autism Diagnostic Interview Revised (caregiver report); ages 30-68 months	Child's sex, total household income in the year prior to the pregnancy, self-reported maternal race/ethnicity, maternal education at delivery, maternal parity, at least one maternal psychiatric condition, maternal smoking in any month during preconception and pregnancy, and maternal age at birth	< 1 drink per week in month 1 or month 2 was not associated with ASD; 1-2 drinks in month 1 were associated with lower odds of ASD (1-2 drinks in month 2 not significant).

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/ Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Skogerbø et al. (2012)[31]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 1,628)	Denmark 1997-2003	1 to 4 drinks per week	5 to 8 drinks per week	12 g pure alcohol	Interview at 17 weeks	Behavior Rating Inventory of Executive Function parent and teacher forms; age 5	Parental education, maternal IQ, prenatal maternal smoking, child's age at testing, child's gender, maternal binge drinking, maternal age, parity, maternal marital status, family home environment, postnatal parental smoking, pre-pregnancy maternal BMI, health status of child	No association existed between low or moderate PAE and executive function.
Skogerbø et al. (2013)[30]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 1,628)	Denmark 1996-2002	1 to 4 drinks per week	N/A	12 g pure alcohol	Interview at 17 weeks	Parent- and teacher-rated SDQ, age 5	Maternal binge drinking, parental education, maternal IQ, prenatal maternal smoking, child's age at testing, child's gender, maternal age, parity, maternal marital status, family-home environment, postnatal parental smoking, pre-pregnancy maternal BMI, and child's health status	No association existed between low to moderate alcohol consumption and offspring behavior.
Underbjerg et al. (2012)[28]	Danish National Birth Cohort Lifestyle During Pregnancy Study (*n* = 1,628)	Denmark 1997-2003	1 to 4 drinks per week	5 to 8 drinks per week	12 g pure alcohol	Interview at 17 weeks	Test of Everyday Attention for Children at Five, age 5	Parental education, maternal IQ, maternal smoking in pregnancy, child's age at testing, gender, and tester were considered core confounding factors, whereas the full model also controlled the following potential confounding factors: maternal binge drinking or low to moderate alcohol consumption, age, BMI, parity, home environment, postnatal smoking in the home, child's health status, and indicators for hearing and vision impairments	No association existed between low or moderate PAE and attention.

Appendix 1. Studies of Low to Moderate Prenatal Alcohol Exposure and Neurodevelopmental Outcomes (Continued)

Author (Year)	Data Source (*n*)	Setting; Offspring Birth Years	Dose of PAE: Low/Light	Dose of PAE: Moderate	Definition of One Drink/Unit of Alcohol	PAE Measurement	Outcome; Offspring Age at Measurement	Covariates	Finding
Weile et al. (2020)[24]	Aarhus Birth Cohort (*n* = 48,072)	Denmark 1998-2012	Analyzed as categories: < 1, 1, 2, or ≥ 3 drinks per week	N/A	12 g pure alcohol	Questionnaire in early pregnancy (median 11 weeks)	ADHD diagnosis from Danish health registries, median age 12 (up to age 19)	Maternal age, highest attained educational level, chronic disease, presentational BMI, smoking in pregnancy, parity, birth year, binge drinking	Up to 1 drink per week PAE was associated with lower risk of ADHD; no associations existed with 2 or more drinks per week.
Williams Brown et al. (2010)[45]	Early Childhood Longitudinal Studies—Birth Cohort (*n* = 10,700)	United States 2001	Low to moderate: 0 to 4 drinks per week Analyzed in categories < 1 drink per week, 1-3 drinks per week		Not reported	Maternal report at 9 months postpartum (range 6 to 22 months)	Bayley Short Form—Research Edition; Nursing Child Assessment Teaching Scale, Behavior Rating Scale, Infant/Toddler Symptom Checklist; age 9 months	Race, poverty, child's age at assessment	< 1 drink or 1 to 3 drinks were not associated with most outcomes including Bayley Mental or Motor subscales, sensory regulation variables, and behavior rating scale. < 1 drink and 1 to 3 drinks were associated with undesirable social engagement and child interaction.

Note: AA, absolute alcohol; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; BAS, British Ability Scale; BMI, body mass index; BSID, Bayley Scales of Infant Development; BSID-III, Bayley Scales of Infant and Toddler Development, third edition; CBCL, Child Behavior Checklist; dl, deciliter; g, grams; K6 score, Kessler Psychological Distress Scale score; K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Age Children; MDI, mental development index; ml, milliliter; N/A, not applicable; NEPSY-II, NEuroPSYchological assessment, second edition; NIH, National Institutes of Health; PAE, prenatal alcohol exposure; PDI, psychomotor development index; *SD*, standard deviation; SDQ, Strength and Difficulties Questionnaire; SES, socioeconomic status; T1, first trimester; T2, second trimester; T3, third trimester.

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Alcohol's Negative Emotional Side: The Role of Stress Neurobiology in Alcohol Use Disorder

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This article is a summary of the presentation delivered at the NIAAA 50th Anniversary Science Symposium on December 1, 2020. It serves as a tribute to NIAAA in commemoration of their persistent commitment to developing the science of alcohol effects and associated harm, and to developing novel cutting-edge strategies in support of prevention and treatment of, and recovery from, alcohol use disorder. I was honored to present at this symposium that captured some of the innovative research supported by NIAAA over the years. It is especially personally meaningful as the discoveries presented here would not have been possible without the financial and intellectual support provided by NIAAA and its dedicated staff to my work and lab over the past 25 years. It has been a real privilege to receive this support from NIAAA to conduct this work and to have this opportunity to share the research findings at this important symposium.

Disclosures
The author declares no competing financial or nonfinancial interests.

Publisher's Note
This article was based on a presentation at the NIAAA 50th Anniversary Science Symposium, "Alcohol Across the Lifespan: 50 Years of Evidence-Based Diagnosis, Prevention, and Treatment Research," held on November 30–December 1, 2020. Links to the videocast are available on the NIAAA 50th Anniversary Science Symposium agenda webpage. Opinions expressed in contributed articles do not necessarily reflect the views of NIAAA, National Institutes of Health. The U.S. government does not endorse or favor any specific commercial product or commodity. Any trade or proprietary names appearing in *Alcohol Research: Current Reviews* are used only because they are considered essential in the context of the studies reported herein.

This article is part of a Festschrift commemorating the 50th anniversary of the National Institute on Alcohol Abuse and Alcoholism (NIAAA). Established in 1970, first as part of the National Institute of Mental Health and later as an independent institute of the National Institutes of Health, NIAAA today is the world's largest funding agency for alcohol research. In addition to its own intramural research program, NIAAA supports the entire spectrum of innovative basic, translational, and clinical research to advance the diagnosis, prevention, and treatment of alcohol use disorder and alcohol-related problems. To celebrate the anniversary, NIAAA hosted a 2-day symposium, "Alcohol Across the Lifespan: 50 Years of Evidence-Based Diagnosis, Prevention, and Treatment Research," devoted to key topics within the field of alcohol research. This article is based on Dr. Sinha's presentation at the event. NIAAA Director George F. Koob, Ph.D., serves as editor of the Festschrift.

KEYWORDS: alcohol; distress; craving; relapse; negative emotions; neural activity; glucocorticoids

The word "alcohol" often conjures up positive feelings and associations with fun, socializing, relaxing, and partying. Yet there is another side to drinking alcohol, especially with risky, hazardous levels of consumption. This side is associated with distress and may include anxiety, loneliness, pain, and depressive symptoms.[1] This has been labeled the "dark side," or "negative emotional, stress side," of alcohol intake.[2] These two paradoxical, dialectically opposing alcohol experiences map onto the biphasic drug effects of alcohol, with alcohol being both a stimulant and a depressant drug. They also represent a shift from positive to negative situations that may drive alcohol intake, especially as alcohol intake increases from low or moderate "social" levels of drinking to binge, heavy, and chronic consumption. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines drinking in moderation as an intake of two drinks or less per day for men and one drink or less per day for women. Binge drinking is generally defined as five or more drinks per occasion for men and four or more drinks per occasion for women. Heavy drinking is generally defined as more than four drinks per day or more than 14 drinks per week for men and as more than three drinks per day or more than seven drinks per week for women.[3]

One aspect of the research the author has conducted with the support of NIAAA, and which is the topic of this article, has focused on identifying the physiological and neural effects, as well as the subjective and cognitive effects, of binge and chronic alcohol use. This research also has explored the factors that influence these effects and investigated whether these effects can be reversed or normalized to allow for recovery from any of the long-term changes that occur with binge and chronic alcohol misuse.

The worldwide coronavirus (COVID-19) pandemic is a chronic, ongoing stressor. Research has shown that alcohol consumption has increased significantly during this period, especially among individuals who regularly binge drink or drink heavily.[4,5] While onsite alcohol sales were down as businesses closed, e-commerce profits increased more than 30% during the COVID-19 pandemic.[4,5] Who is most susceptible to increased drinking episodes during COVID-19–related stress? This question highlights the need to understand the well-known bidirectional relationship between stress or trauma and alcohol intake, and why those with binge and chronic alcohol use are most vulnerable to increased alcohol use under high levels of stress and with traumatic exposure.

This article reviews human research investigating neurobiological and psychological changes related to alcohol misuse that are associated with greater distress and stress-related alcohol craving and their role in predicting risk of binge drinking, relapse, and impact on treatment outcomes. The author presents the effects of stress and trauma on brain stress responses and their associations with resilient coping and describes the impact of binge and chronic alcohol use on brain and peripheral stress responses and their role in promoting alcohol craving and relapse risk. Specific clinical and biobehavioral markers of both risk of developing alcohol use disorder (AUD) and relapse are also reviewed. Finally, the article discusses recent findings on treatments that focus on reversing stress and craving disruptions related to chronic alcohol misuse to improve treatment outcomes.

Alcohol and Stress—Shift From Positive to Negative Effects

It is well known that one or two standard alcoholic drinks have a stimulating and physiologically arousing effect; for example, heart rate increases acutely, and blood pressure changes have been documented. These responses are part of the autonomic nervous system readouts that occur with alcohol intake, but also are observed in challenging situations such as when faced with acute stressful life events.[6,7] The arousing response to alcohol is associated with a sense of feeling energized and stimulated as well as increases in sociability.[6] With increasing levels of alcohol intake in one sitting, however, alcohol also stimulates the hypothalamic-pituitary-adrenal (HPA) axis, and increases in cortisol are observed.[8,9] Alcohol also activates brain emotion and stress pathways, including the amygdala, under emotional arousing and stressful states.[10,11] In addition, acute alcohol use stimulates the brain cortico-striatal pathways involved in reward, motivation, and goal-directed behaviors. These include the ventral and dorsal striatum, the orbitofrontal cortex (OFC), and the ventromedial prefrontal cortex (VmPFC).[10-13] The emotion/stress pathway and the reward/motivation pathways closely interact, and such interactions are involved in emotional cue-related drinking motivation.[11,12]

Binge and hazardous alcohol drinking patterns are associated with well-documented changes both in the brain stress and emotion regions, such as the amygdala,[8,12] and in associated brain networks, including the ventral and dorsal striatum as well as the OFC, VmPFC, and dorsolateral prefrontal cortex.[9,12,14,15] These brain changes are associated with blunted autonomic and cortisol responses to stress and to acute alcohol intake,[6,8] as well as with increases in negative emotional and stress responses and greater alcohol craving.[6,9,14-17] Together, these changes are part of the psychobiological adaptations in humans that occur with increasing patterns of binge and hazardous alcohol intake.

Stress, Alcohol Craving, and Binge Alcohol Intake

Acute stress exposure stimulates the autonomic, endocrine, and brain emotion and motivation regions that process and regulate negative emotion and distress responses, and it also activates

stress coping.[6,12,18] Additionally, acute stress exposure increases physiological arousal, including cortisol responses, and activates brain stress pathways involved in emotional arousal, emotional learning, and memory. This activation occurs via circuits involving the hypothalamus, amygdala, hippocampus, insula, and prefrontal regions, including the OFC, VmPFC, and inferior frontal cortices. Also activated is the premotor supplementary motor area, which is involved in behavioral intent, response selection, and action.[6,18,19] Previous studies reported that there are dynamic time-dependent changes in the cortico-striatal regions involving the ventral and dorsal striatum and the VmPFC during stress versus non–stress conditions; these changes were associated with active, goal-directed stress coping.[18] Additionally, greater dynamic responses in these brain stress-reward pathways were associated with lower daily numbers of alcoholic drinks consumed, lower reports of emotional conflicts, and lower emotional eating, whereas blunted ventral striatum and VmPFC responses during stress were associated with greater reports of binge drinking, emotion dysregulation, and emotional eating.[18] Based on these findings, the dynamic neural responses in the striatum and VmPFC are thought to document neurophysiological flexibility during stress, and their associations with behavioral coping suggest that this circuit is part of the resilient stress-coping pathway involved in behavioral control and self-regulation of stress, emotions, and reward impulses.[6,18]

These adaptations to alcohol also vary by sex, as fundamental differences between men and women exist in brain organization, structure, and functional networks[20] as well as in the responses of brain stress, emotion, and reward regions[21] and in patients with cocaine use disorder.[22] Moreover, sex differences in the responses to stress and to alcohol-related stimuli have been documented in people who drink moderately. Unlike in animal studies, males in human studies show greater adrenocorticotropic hormone (ACTH) and cortisol responses to stress,[23] whereas females show higher autonomic physiologic arousal to stress; a greater response to stress cues in the amygdala, insula, OFC, and VmPFC; and greater VmPFC response to alcohol cues.[24-28] This suggests that the psychological and biological responses to alcohol and to stress vary by sex and that although men and women report similar levels of alcohol motivation when matched for recent drinking history, the psychological and neurobiological pathways that facilitate alcohol use are different for men and women who drink moderately.

Regardless of sex, repeated escalated alcohol use induces changes in both peripheral and brain stress systems.[2,12,16] Higher binge levels of alcohol use increase basal cortisol levels and blunt the peripheral stress responses; these changes also predict greater craving and behavioral motivation for alcohol use in people who binge drink or drink heavily (see Figure 1).[8,9] Additionally, changes in the amygdala responses to emotional cues and ventral striatal responses to alcohol have been reported with higher binge levels of alcohol use.[14,29] Along with these neural changes, increased salience of alcohol and greater alcohol craving levels have been observed in response to stress as well as in response to alcohol and to alcohol cues, which then promote increased alcohol intake and escalation to risky drinking.[8,15,17] These brain stress system, physiologic, and behavioral effects of binge drinking history need to be further examined by sex to better understand the recent data on greater escalation of binge drinking in women compared to men.[30]

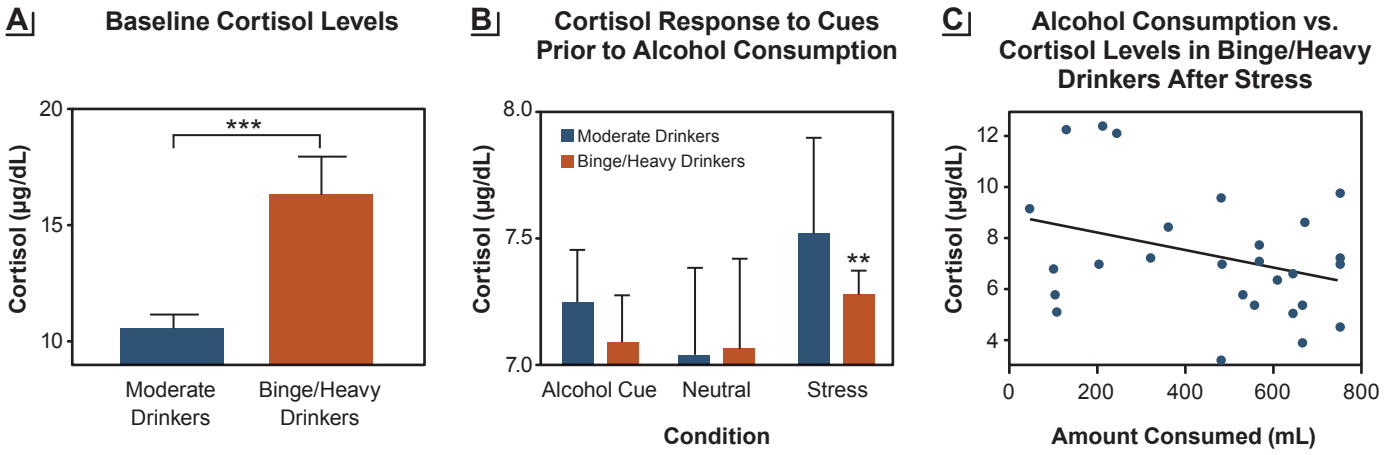

Figure 1. Baseline cortisol levels and responses to stress differ between moderate drinkers and binge/heavy drinkers. (A) Fasting morning plasma levels of cortisol (µg/dL) were higher in binge/heavy drinkers (orange bars) compared to moderate drinkers (blue bars) (***$p < .001$). (B) Cortisol responses to stress and alcohol cues, but not to neutral cues, were blunted in binge/heavy drinkers compared with moderate drinkers (**$p < .01$). (C) In binge/heavy drinkers, the behavioral motivation for alcohol use as reflected in the amount of alcohol consumed post stress in an ad lib drinking task was greater in individuals with a more blunted cortisol response to stress ($r^2 = .11$, $p = .0022$). *Source:* Adapted with permission from Blaine et al. (2019).[8]

Effects of Stress and Trauma on Brain Pathways and AUD Risk

Stress and trauma are associated with greater levels of risky alcohol intake as well as greater severity of AUD.[19] Numerous different types of traumatic stress and life events as well some temperament and individual-level variables relate to risk of binge drinking and developing AUD (see Table 1). Exposure to repeated stress and trauma also contributes to changes in the brain and body's responses to stress and emotions as well as to changes in alcohol motivation and adaptive coping responses.

Greater levels of cumulative adversity, stressful life events, and trauma are associated with lower brain volume and greater negative emotion and subjective stress responses. They also are associated with dysregulated neural and peripheral physiological responses to stress and to alcohol cues in the brain regions involved in stress, emotion, reward regulation, and self-control, including the OFC, VmPFC, supplementary motor area, amygdala, insula, and striatum.[31-33] Furthermore, altered or blunted ACTH and cortisol and autonomic responses to stress and to alcohol and drug cues are observed with greater trauma or stress.[19,33] These stress- and trauma-related brain and peripheral alterations co-occur alongside emotional and behavioral dysregulation and higher alcohol motivation. As a result, people with more risky drinking exposed to stress or trauma are at greater risk of emotion dysregulation as evidenced by more arguments, fights, emotional eating, and higher maximum drinks consumed per occasion (see Figure 2).[18,34]

Several interacting brain networks are activated during stress, including those involved in emotion experiences (e.g., amygdala, insula), emotional memory (e.g., amygdala, hippocampus), reward and motivation regions (e.g., ventral and dorsal striatum), and goal-directed behavior (e.g., OFC, VmPFC).[13,18,19,21,29] These regions form networks and patterns of activation that enable emotional and motivational coping, and both stress and alcohol directly act on these networks to influence active coping, motivation, and flexible control of behavior, such as exercising self-control with drinking. The accumulating evidence shows that stress and trauma exposure alter these emotional and motivational responses involved in adaptive stress coping, such that people become more vulnerable to craving and consuming higher levels of alcohol, which increases risk of hazardous and risky drinking.

The research described above resulted in the development of a model explaining the role of glucocorticoids in drinking behavior on the basis of changes in peripheral cortisol levels and responses across the full spectrum of alcohol consumption levels.[8] At baseline, people who binge drink or drink heavily have higher cortisol levels than those who drink moderately (see Figure 1A), indicating a shift in HPA axis functioning. This also suggests possible changes in brain glucocorticoid pathways in

Table 1. Types of Adverse Life Events, Trauma, Chronic Stressors, and Individual-Level Variables Predictive of Addiction Risk

Adverse Life Events	Childhood and Life Trauma	Chronic Stressors	Stressful Internal States
• Loss of parent • Parental divorce and conflict • Isolation and abandonment • Single-parent family structure • Forced to live apart from parents • Loss of child by death or removal • Unfaithfulness of significant other • Loss of home to natural disaster • Death of significant other or close family member	• Physical neglect • Physical abuse by parent, caretaker, family member, spouse, or significant other • Emotional abuse and neglect • Sexual abuse • Rape • Victim of gun shooting or other violent acts • Observing violent victimization	• Being overwhelmed • Unable to manage life problems • Difficulties with job, living situation • Financial problems • Interpersonal conflicts, loneliness • Unfulfilled desires • Problems with children • Illness of loved ones • Negative emotionality • Poor behavioral control • Poor emotional control	• Hunger or food deprivation • Food insecurity • Extreme thirst • Sleep deprivation or insomnia • Extreme hypothermia or hyperthermia • Excessive drug use • Drug withdrawal states • Chronic illness

Source: Included with permission from Milivojevic & Sinha (2018).[37]

humans that may increase risk of hazardous drinking. As stated earlier, alcohol consumption stimulates cortisol release; however, in response to either stress or alcohol exposure, the increase in cortisol is lower in people who binge drink or drink heavily than in those who drink moderately. Thus, when given one standard alcoholic drink, those drinking at binge levels do not feel its effects as robustly as do people who drink moderately.[8,9] As cortisol is critical for survival, humans have well-preserved neurobehavioral signals with the brain stress system pathways[12] that seek to enhance cortisol release in response to stress. In people with blunted cortisol responses due to heavy drinking, this mechanism may signal greater motivation for alcohol to increase alcohol-related cortisol responses.[9] Thus, there is a neurophysiologic drive to enhance wanting alcohol in order to increase cortisol and HPA axis functioning in people who drink heavily. This disruption in alcohol-related cortisol signaling and the need to drive the homeostatic HPA axis rhythm back to functional levels may be one component of the enhanced motivation for alcohol in those who drink alcohol at binge and heavy levels. This conceptual model suggests that normalizing the brain and body's stress and motivational coping responses may reduce risk of hazardous drinking. Researchers are seeking to develop and evaluate novel strategies to achieve this normalization and to reduce the risk of heavy drinking.

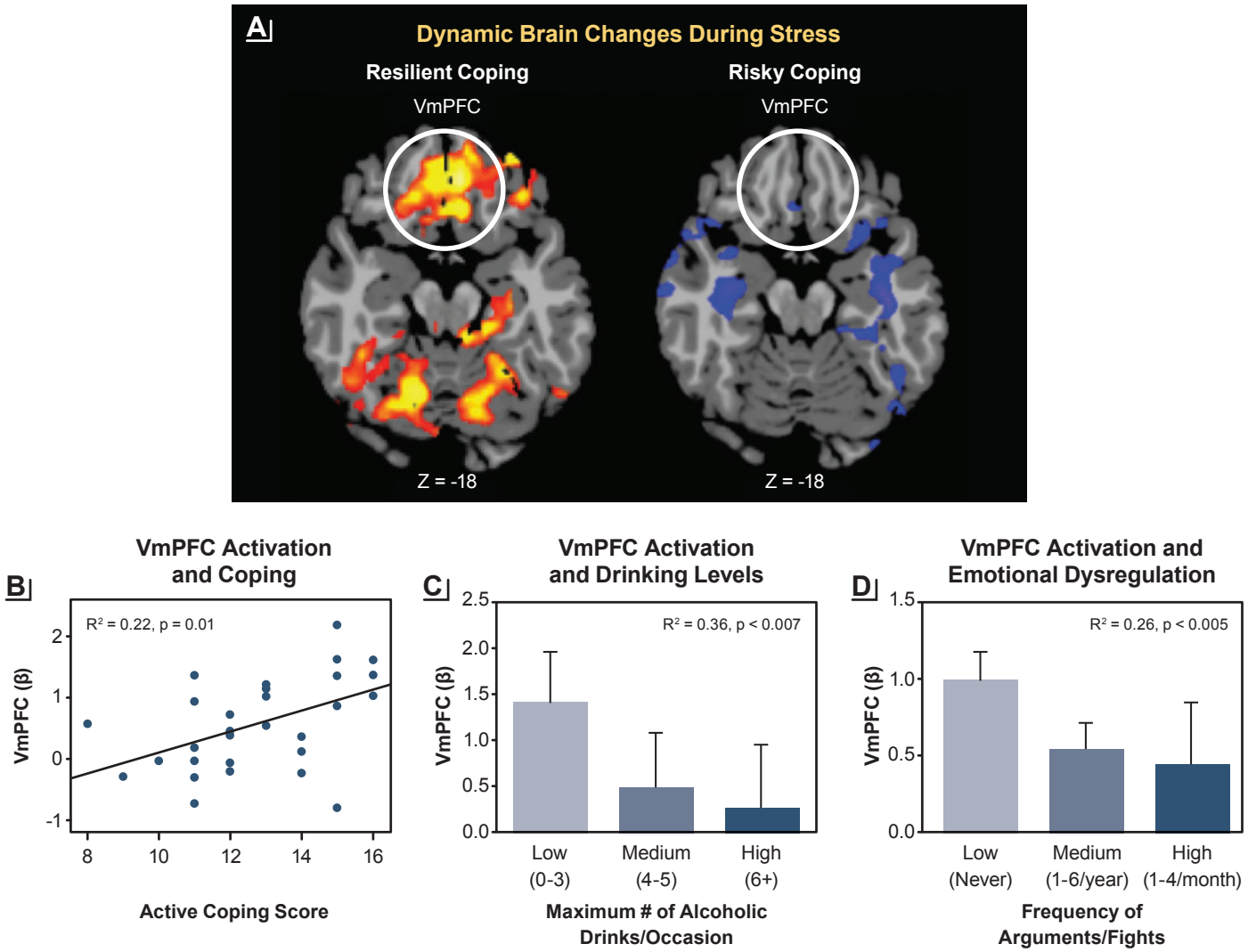

Figure 2. Associations between brain stress responses and resilient coping. (A) Dynamic activation in the ventromedial prefrontal cortex (VmPFC) during stress challenge (represented by red and yellow) was a sign of resilient coping, whereas a lack of dynamic changes in the VmPFC during stress, suggesting inability to mobilize during stress, was a sign of risky coping. (B) Greater dynamic activation of the VmPFC was associated with greater self-reported active coping. (C) Lack of dynamic activation of the VmPFC was more pronounced in binge drinkers. (D) Greater emotional dysregulation (measured by greater frequency of arguments or fights) also was associated with less dynamic activation of the VmPFC. *Source:* Adapted with permission from Sinha et al. (2016).[18]

Effects of Stress and Alcohol Cues in AUD

Researchers also have investigated the role of stress biology and stress responses in people with AUD. Chronic heavy drinking or binge drinking increases the risk of disrupted alcohol-related autonomic and HPA axis responses as described in previous sections. These disruptions contribute to clinical symptoms associated with the negative emotional side of AUD,[15] such as increased levels of anxiety, negative mood, sleep difficulties, emotional reactivity, and impulsivity, along with high levels of craving for alcohol.[1,35] Furthermore, these disruptions increase the risk of relapse and heavy drinking during treatment and posttreatment, thereby jeopardizing long-term recovery.[6,36,37] Alcohol relapse refers to return to heavy drinking (at binge levels) after any period of abstinence, whereas treatment failure refers to maintaining or returning to binge and hazardous drinking levels during or after treatment.[3] These observations have led researchers to investigate which factors contribute to early risk of dropout and recovery failure during treatment.

A series of studies assessed brain and body responses as well as cognitive, emotional, and motivational responses to both stress and alcohol cues in a laboratory study of human participants with AUD who were entering treatment and control participants without AUD. The analyses also included structural and functional magnetic resonance imaging as well as real-world daily assessment of stress and motivational responses using smartphones. These analyses using multiple approaches across different samples of individuals with AUD found that stress exposure increased alcohol craving. This response was accompanied by higher emotional, mood, and anxiety symptoms and lower ability to regulate emotions and control alcohol cravings.[36,37] Furthermore, the biological stress response was significantly disrupted during the early recovery period. Thus, individuals in early recovery exhibited a higher basal heart rate and higher free cortisol levels, but lower levels of endogenous bound cortisol. Additionally, these individuals did not show a significant normal response to stress or alcohol challenge.[6,37] Thus, the biological responses that support emotion and mood regulation are disrupted during this early recovery phase, and the greater these levels of dysfunction, the higher the risk of relapse or heavy drinking. Notably, sex differences in these biological responses have been reported, where women with AUD showed a more blunted ACTH and cortisol level than men with AUD; however, women had much higher basal norepinephrine levels, which in turn affected their response to stress and to alcohol cues.[26,38]

Another series of experiments examined brain correlates of later alcohol relapse and treatment failure. These analyses found that the volume of gray matter cells in the medial prefrontal brain regions—which are involved in regulating emotions, reward, and actions—was lower among individuals entering treatment compared with healthy control participants.[39] Also, individuals with the lowest gray matter volume in the medial prefrontal brain region tended to be most likely to relapse and not do well in treatment.[39] Analyses assessing the function of these brain regions during experimental exposure to stress and to alcohol cues (compared to neutral cues) detected disrupted, hyperactive VmPFC responses to neutral relaxing cues, but blunted, hypoactive VmPFC responses to stress and cue exposure. These observations suggest that the brain pathways that help regulate emotions and desires showed dysfunction and that the greater the VmPFC disruption, the higher the risk of alcohol relapse and heavy drinking.[40,41]

The studies described above have led to the characterization of a risk profile to identify individuals who are most vulnerable for alcohol relapse and heavy drinking during treatment. Thus, risk was determined by specific clinical measures—such as alcohol craving and withdrawal,[42,43] mood, anxiety, and sleep difficulties—and biological markers[37] as well as by additional moderating factors, including childhood maltreatment (see Table 2).[44] Furthermore, this research supported the conceptualization that the effects of binge drinking and chronic alcohol use on stress biology occur along a continuum, with higher levels of alcohol intake associated with more significant chronic stress pathophysiology, which in turn contributes to greater risk of alcohol relapse and treatment failure.[35]

AUD Treatments Targeting Stress, Craving, and Loss of Control of Alcohol Intake

Critical basic science and translational work by Koob and colleagues[45] had focused on stress pathophysiology to develop novel therapeutics for AUD. Similarly, the findings described above motivated additional research to evaluate whether reversal of the chronic alcohol-related disruptions in stress psychobiology that are associated with increased alcohol craving and relapse risk could improve treatment and treatment outcomes for individuals most vulnerable to alcohol-related stress pathophysiology. Previous research by Arnsten had shown that noradrenergic agents such as guanfacine and prazosin could rescue the prefrontal cortex from the toxic effects of high uncontrollable stress.[46] Because the effects of chronic alcohol exposure are similar to those of high chronic stress, it seemed plausible that pharmacologic targets that reduce prefrontal norepinephrine and the toxic effects of stress-related damage also could be of benefit in improving the stress and craving-related pathology associated with AUD. Studies to test these hypotheses have shown positive results. Guanfacine, an alpha-2 adrenergic agonist that reduces brain norepinephrine in the prefrontal cortex, improved prefrontal functioning and reduced alcohol and drug craving.[47,48]

Table 2. Markers and Moderators Associated With Relapse to Alcohol Use and Treatment Failure in Alcohol Use Disorder (AUD)

Clinical and Biological Markers	Moderating Factors
• Increased levels of alcohol craving • High early physical, sexual, emotional abuse and trauma history • High basal beat-by-beat heart rate and blunted autonomic response to stress and cues • Altered bound and free fasting morning cortisol levels, and adrenal sensitivity • Blunted and hypoactive cortisol response to stress • Lower medial prefrontal gray matter volumes in magnetic resonance imaging • Blunted medial prefrontal cortex response to stress and alcohol cues • Hyperactive striatal responses to alcohol cues	• AUD severity, including life span factors of early or late AUD; acute withdrawal symptoms, including anxiety, sleep, and negative mood; alcohol abstinence days • Early physical, sexual, and emotional abuse and lifetime traumas; chronic stress; and trauma-related pathophysiology • Sex differences and gender-related comorbid psychopathology and medical conditions • Genetic and pharmacogenomic effects

Furthermore, guanfacine had some sex-specific effects, with greater benefits in women than in men.[49,50]

Similarly, prazosin—an alpha-1-adrenergic antagonist that had been shown to improve working memory and prefrontal functioning during stress[46] as well as withdrawal-related drinking in laboratory animals[51]—reduced stress-related craving and stress dysfunction in AUD.[52,53] Based on these findings, an NIAAA-supported, 12-week proof-of-concept, double-blind, placebo-controlled, randomized trial of prazosin versus placebo (16 mg/day, three times a day dosing, titrated over 2 weeks) was conducted with 100 individuals with AUD. The study found that alcohol withdrawal symptoms were a moderating factor impacting prazosin efficacy in improving drinking outcomes over 12 weeks; that is, prazosin treatment benefit was determined by the presence of alcohol withdrawal symptoms at treatment entry. Thus, individuals with more severe alcohol withdrawal symptoms at treatment initiation experienced greater reductions in heavy drinking days and drinks per occasion during the 12-week treatment period.[54] In addition, prazosin reduced alcohol craving, anxiety, and negative mood compared with placebo in participants with high alcohol withdrawal symptoms, but had no impact in those with no or low levels of alcohol withdrawal symptoms. Finally, prazosin appeared to reverse VmPFC and dorsal striatal dysfunction, improving medial prefrontal response to stress and reducing dorsal striatal response to alcohol cues in participants treated with prazosin compared with those receiving placebo.[55] These findings support further development of prazosin in the treatment of severe AUD. However, they also underscore the need to pursue further research to identify behavioral and pharmacologic strategies to prevent and treat chronic alcohol effects on stress pathophysiology in AUD.

Conclusions

This article summarizes research by the author's group demonstrating that binge, heavy, and chronic drinking leads to adaptations in brain, biological, and psychological stress responses. These adaptations are associated with alcohol's negative emotional aspects, as evidenced by greater alcohol craving, higher alcohol withdrawal, greater negative mood and anxiety symptoms, as well as sleep difficulties that are commonly reported by individuals with AUD entering treatment. These changes occur in brain stress, reward, and motivation pathways that represent the stress pathophysiology of AUD. This stress pathophysiology directly targets brain circuits that underlie people's ability to cope with stress and day-to-day challenges and are involved in jeopardizing recovery from AUD.

This research also has identified various clinical and biobehavioral markers that are associated with relapse and treatment failure and has allowed for identification of individuals who may be at greatest risk of treatment failure. Additionally, identification of these markers has led to research seeking to develop new strategies to target and reverse the stress pathophysiology of AUD to optimize interventions for AUD. Current and future work is focused on developing and testing specific treatments that can target this particular stress pathophysiology and help individuals who are most vulnerable to jeopardizing their recovery in the early phase of AUD treatment.

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Alcohol-Endocannabinoid Interactions: Implications for Addiction-Related Behavioral Processes

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PURPOSE: The endogenous cannabinoid system is involved in several physiological functions in the central nervous system including the modulation of brain reward circuitry and emotional homeostasis. Substantial evidence implicates brain endocannabinoid signaling in the processing of drug-induced reward states, wherein repeated exposure besets pathological changes in activity that contribute to the progression of alcohol use disorder. This review provides a narrative summary of recent studies exploring the interaction between alcohol exposure and changes in endocannabinoid signaling that may underlie the development of alcohol use disorder.
SEARCH METHODS: The authors began with an initial search for review articles to assist in the identification of relevant literature. This was followed by separate searches for primary literature and recent studies. The search terms "alcohol/ethanol" and "endocannabinoids" were applied, along with terms that covered specific objectives in reinforcement and addiction behavior. The content was further refined by excluding articles containing a broad focus on psychiatric disorders, polysubstance abuse, non-cannabinoid signaling lipids, and other criteria.
SEARCH RESULTS: The initial search yielded a total of 49 review articles on PubMed, 13 on ScienceDirect, and 17 on Wiley Online, from which the authors garnered information from a total of 16 reviews. In addition to independent searches, this review provides information from a collection of 212 publications, including reviews and original research articles.
DISCUSSION AND CONCLUSIONS: The review discusses the effects of alcohol consumption on brain endocannabinoid signaling, including alcohol-based perturbations in endocannabinoid-mediated synaptic transmission, the modulation of alcohol-related behaviors by manipulating signaling elements of the endocannabinoid system, and the influence of dysregulated endocannabinoid function in promoting withdrawal-induced anxiety-like behavior. Notable emphasis is placed on studies exploring the possible therapeutic relevance of bolstering brain endocannabinoid tone at different stages of alcohol use disorder.

KEYWORDS: alcohol; dependence; cannabinoids; anxiety; reinforcing; anandamide; 2-arachidonoylglycerol; effects on the brain

Endogenous cannabinoids, or endocannabinoids (eCBs), are bioactive lipid molecules that modulate signaling activity of several physiological processes involved in pain, appetite, energy balance, stress/anxiety, immune signaling, and learning and memory. Although understanding of the eCB system has grown in complexity since its discovery by Raphael Mechoulam, it is now widely known that eCB systems play an important role in the regulation of brain reward and emotional homeostasis. Given the relevance of these physiological responses in motivated behavior, the hypothesis of the involvement of eCB systems in addiction has been widely investigated.[1-3] Generally, these findings support a role for eCB signaling in mediating the positive reinforcing effects of substances with abuse potential, while repeated drug exposure elicits long-lasting changes aligned with the emergence of negative affective states during abstinence. While these changes ostensibly apply to more than one type of substance with abuse potential, the field has come to understand the strong relation between negative affective states and increased alcohol consumption that facilitates the development of alcohol use disorder (AUD).[4] Extensive efforts have been made to study the role of eCB systems in alcohol-induced pathologies.[5,6] Highlighted here is recent work exploring the basis of alcohol-eCB interactions in the development of AUD. A brief overview of the molecular constituents involved in eCB synthesis and degradation is followed by a foray of the literature exploring the effect of alcohol consumption on brain eCB signaling. Emphasis is placed on cutting-edge research utilizing genetic and pharmacological approaches to discretely manipulate elements of eCB signaling. This review discusses these findings in terms of the purported roles of the eCBs in synaptic plasticity, stress, and anxiety, and further elucidates the therapeutic relevance of bolstering brain eCB tone in the possible treatment of AUD.

Search Methods and Results

Searches of the existing literature were primarily conducted on PubMed/PubMed Central. The authors first conducted a broad search of review articles to assist in the identification of primary literature. The terms "alcohol" or "ethanol" and "endocannabinoid" were searched, restricted to the "title/abstract" setting under the "Advanced Search Builder" function. The authors then activated search filters for "Reviews" published within 10 years of June 2021. This search strategy led to the identification of 49 review articles. Similar search strategies in ScienceDirect and Wiley Online Library generated fewer citations (13 and 17, respectively), the majority of which were redundant. To narrow the search more specifically to the goals of the current work, the authors excluded reviews with a broad focus on psychiatric disorders or polysubstance use, fetal drug exposure, non-cannabinoid signaling lipids, phytocannabinoids and other metabolites, as well as eCB/cannabinoid responses outside of the central nervous system. Thus, the authors conducted a thorough reading of 16 reviews.

Separate searches were then conducted to identify primary literature and recent studies using the terms "alcohol/ethanol" and "endocannabinoid" along with general terms covered in each section of the review (e.g., "reward," "consumption," "withdrawal/abstinence," "dependence," "anxiety," "FAAH inhibitors," "MAGL inhibitors"). In some cases, this article refers to reviews and primary literature from major contributors in the field or from the respective laboratories of the authors of this review. All searches were restricted to the English language and generally reflect published work from 1990 to the present, with a few exceptions for foundational work on lipid-alcohol interactions. Most of the studies presented here concern data collected in rodent models. For information on clinical trial testing, the clinicaltrials.gov website was used. This review cites information from a total of 212 publications.

The eCB System

The eCB system comprises two G-protein coupled receptors, their endogenous lipid ligands, and the enzymes that mediate synthesis and clearance of these molecules. Currently, there are two major types of cannabinoid receptors that are well characterized and cloned: cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2). CB_1 receptors are mainly found on presynaptic terminals of neurons in the brain,[7,8] whereas CB_2 receptors are mostly expressed in immune cells of peripheral tissues,[9] but are also found in the central nervous system.[10-13] Both receptors are coupled to $G_{i/o}$ protein second messenger systems regulating the amount of cyclic adenosine monophosphate levels in the cell and, by extension, the concentration of intracellular calcium and potassium ions that facilitate synaptic transmission. The relative importance of CB_1 versus CB_2 signaling is still under investigation; however, CB_1 receptors are abundantly found in mesocorticolimbic areas that are important for reward and motivation.[2,14]

Currently, the best-studied endogenous ligands of cannabinoid receptors are two arachidonic acid derivatives, *N*-arachidonylethanolamine (anandamide or AEA) and 2-arachidonylglycerol (2-AG). Several other endogenous compounds possess cannabinoid-like properties, although much regarding their pharmacological activity, synthesis, and metabolism remains to be characterized.[15] AEA and 2-AG activate cannabinoid receptors with a high degree of specificity (see Figure 1A). AEA is a partial agonist of both cannabinoid receptors, with slightly higher affinity for CB_1 than CB_2 receptors. On the other hand, 2-AG is a full agonist of both receptors, exhibiting low to moderate affinity for each subtype, and with greater overall potency and efficacy than AEA.[15,16] AEA and 2-AG demonstrate some promiscuity to other receptor systems,

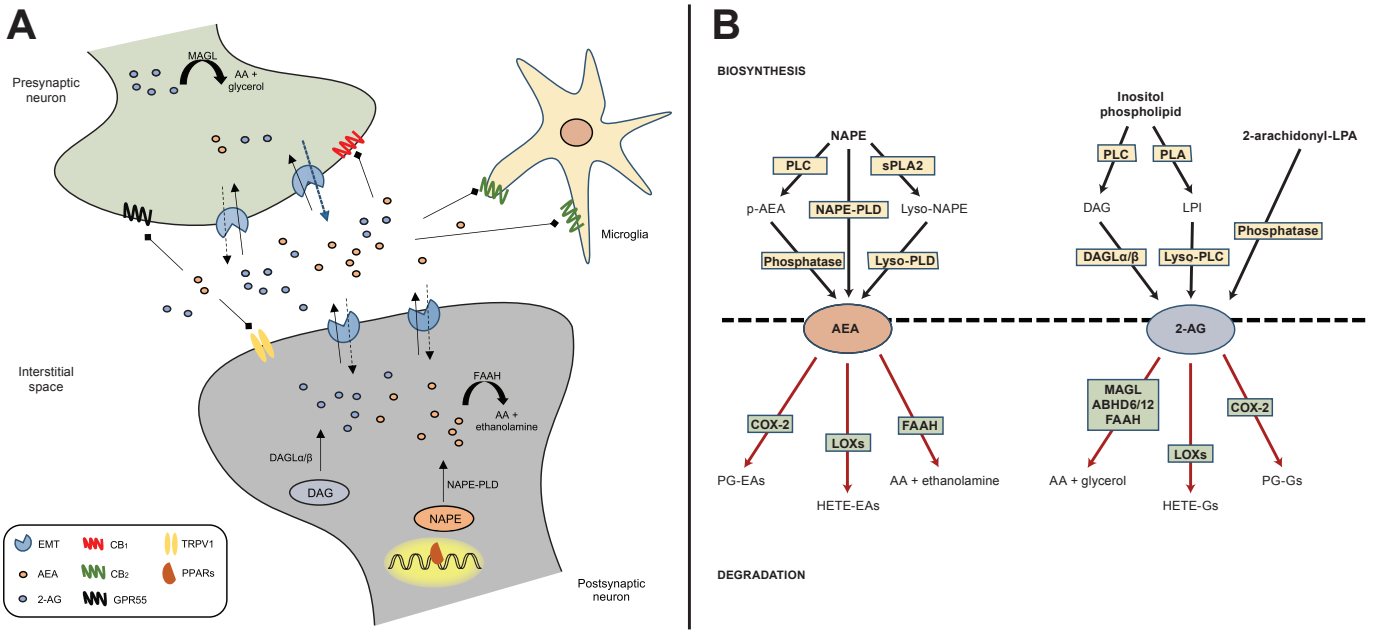

Figure 1. Endocannabinoid signaling and biosynthetic/degradation mechanisms. A: Schematic representation of the synaptic organization of the main components of the endocannabinoid system, including established routes of AEA and 2-AG metabolism. B: Metabolic pathways of synthesis and degradation of AEA and 2-AG. See text for details. *Note:* 2-AG, 2-arachidonylglycerol; 2-arachidonoyl-LPA, 2-arachidonoyl-*sn*-glycero-3-phosphate; AA, arachidonic acid; ABHD6/12, alpha/beta-hydrolase domains 6 and 12; AEA, anandamide; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; COX-2, cyclo-oxygenase 2; DAG, diacylglycerol; DAGLα/β, diacylglycerol lipase-alpha/beta; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; GPR55, G-protein coupled receptor 55; HETE-EAs, hydroxyeicosatetraenoyl-ethanolamides; HETE-Gs, hydroxyeicosatetraenoyl-glycerols; LOXs, lipoxygenases; LPI, lysophosphatidylinositol; lyso-NAPE, lyso-*N*-arachidonoyl-phosphatidylethanolamine; lyso-PLC, lyso-phospholipase C; lyso-PLD, lyso-phospholipase D; MAGL, monoacylglycerol lipase; NAPE, *N*-arachidonoyl-phosphatidylethanolamine; NAPE-PLD, *N*-arachidonoyl-phosphatidylethanolamine-specific phospholipase D; p-AEA, phospho-anandamide; PG-EAs, prostaglandin-ethanolamides; PG-Gs, prostaglandin-glycerols; PLA, phospholipase A; PLC, phospholipase C; PPARs, peroxisome proliferator-activated receptors; $sPLA_2$, soluble phospholipase A_2; TRPV1, transient receptor potential vanilloid type-1.

including peroxisome proliferator-activated receptors (PPARs) and the orphan G-protein coupled receptors 55 (GPR55) and 119 (GPR119).[17-20] AEA is also known for exerting potent agonist effects on transient receptor potential vanilloid type 1.[21]

Unlike classical neurotransmitters, eCBs are not stored in intracellular compartments but instead are produced "on demand" from membrane lipid precursors in the postsynaptic membrane (see Figure 1B). AEA is produced from the phospholipid precursor *N*-arachidonoyl-phosphatidylethanolamine (NAPE) by a NAPE-specific phospholipase D (NAPE-PLD).[22] Interestingly, knockdown of NAPE-PLD only moderately depletes AEA signaling pools, suggesting that AEA contains several redundancies in its biosynthesis.[23] On the other hand, 2-AG is tightly coupled to the production of diacylglycerol from the hydrolysis of an inositol phospholipid by a phospholipase C, which is rapidly converted to 2-AG by two *sn-1*-specific diacylglycerol lipase (DAGL) isoforms (DAGL-alpha and DAGL-beta).[24,25] Emerging research suggests that 2-AG, although widely regarded as the primary synthase, also may be influenced by alternative biosynthetic pathways. One pathway involves the hydrolysis of phosphatidylinositol by a phospholipase A to form a lysophosphatidylinositol, which is hydrolyzed to 2-AG by phospholipase C.[26] Another alternative pathway is by the dephosphorylation of arachidonic acid-containing lysophosphatidic acid by a phosphatase.[27]

Once released into the synaptic cleft, AEA and 2-AG exert their effects through the retrograde activation of CB_1 receptors located on presynaptic terminals, followed by rapid termination of signaling via multiple degrading enzymes. In this regard, AEA is primarily degraded by fatty acid amide hydrolase (FAAH) into free arachidonic acid and ethanolamine,[28] whereas monoacylglycerol lipase (MAGL) is the main enzyme involved in the hydrolysis of 2-AG to produce arachidonic acid and glycerol.[29] Interestingly, these clearance enzymes are located in different cellular compartments. FAAH is mainly localized to the postsynaptic cell, suggesting a key role for this enzyme in monitoring interstitial AEA concentrations. By contrast, MAGL is mainly found in the presynaptic terminal and contributes to the inactivation of 2-AG near its site of action.[30] This configuration would suggest that AEA and 2-AG assume different roles in eCB signaling despite the signaling redundancy to cannabinoid receptors. The enzymatic clearance of 2-AG is mostly driven by MAGL,[31] although other enzymes such as alpha/beta-hydrolase domains 6 and 12

(ABHD6/12)[31,32] and FAAH[33] have been shown to metabolize 2-AG under certain conditions. AEA and 2-AG also may be oxidized by cyclo-oxygenase 2 and several lipoxygenases[34,35] contributing to the pool of liberated arachidonic acid moieties that can be targeted for eicosanoid production. Overall, these metabolic enzymes play a key role in the production and maintenance of AEA and 2-AG signaling, which portend downstream effects on the regulation of the chemical synapse.

Neurochemical Role of eCBs in Synaptic Plasticity

The role of the eCB system in synaptic plasticity largely stems from the findings that stimulation of cannabinoid receptors modulates the release of neurotransmitters at excitatory and inhibitory synapses. Further research has characterized the importance of eCB signaling in providing inhibitory control of fast-acting transmitters such as glutamate and gamma-aminobutyric acid (GABA), as well as in modulating activity of other small molecules, such as mesolimbic dopamine.[36] More generally, eCBs contribute to the shaping of synaptic activity in mesocorticolimbic areas of the brain, which—depending on the strength, frequency, and duration of transmission—can have both immediate and long-lasting consequences on synaptic function.[37-42]

Triggering eCB-CB_1 receptor signaling results in short-term adjustments in neurotransmitter release that modulate activity of the postsynaptic cell via depolarization-induced suppression of excitation or inhibition.[43-45] These transient forms of plasticity typically last a minute or less and are more strongly associated with 2-AG than AEA signaling, although both lipids have been implicated in such responses.[42] Activation of eCB-CB_1 receptor signaling can also facilitate more persistent forms of synaptic plasticity, such as long-term depression (LTD). These events vary with the nature of synaptic stimulation but generally persist anywhere from hours to weeks.[42] The eCB system has long been observed to mediate plasticity in brain regions involved in the etiology of addiction, including the ventral tegmental area, nucleus accumbens (NAc), prefrontal cortex (PFC), hippocampus, amygdala, and dorsal striatum.[1,42,46] In this regard, several conceptualizations of addiction theory propose that drug and alcohol exposure result in the disruption of plasticity mechanisms involved in learning and memory, which may contribute further to maladaptations in brain reward circuitry.[47-49]

Acute and chronic alcohol exposure disrupts eCB-mediated synaptic plasticity. In this regard, low- to moderate-frequency stimulation of the dorsolateral striatum results in the elevation of eCB levels, which is thought to shift the balance of excitatory and inhibitory regulation of striatal neurons toward long-lasting disinhibition of synaptic output.[50] Interestingly, acute alcohol exposure impairs this eCB-mediated process and further reduces LTD of medium spiny neurons at inhibitory relative to excitatory synapses.[51,52] The disruption in eCB function is significant given that neural circuits in the dorsal striatum mediate behavioral processes related to reward-guided learning and habitual responding.[53] In this regard, mice undergoing chronic intermittent alcohol vapor exposure exhibit impaired CB_1-dependent LTD in the dorsolateral striatum that corresponded with increases in dorsolateral striatal activation and enhanced stimulus-reward learning.[54] More recently, intermittent alcohol exposure during adolescence conferred long-lasting impairments in CB_1-dependent LTD in the hippocampus that were associated with disruptions in recognition memory.[55] These findings suggest that alcohol dysregulates eCB signaling in a manner that fundamentally changes the regulation of the chemical synapse. Impairments in eCB-mediated plasticity likely reflect the loss of an important source of inhibitory constraint of neuronal synapses, leading to pathology in reward-based learning and the modulation of rewarded behavior that influences the progression of AUD.

Alcohol-Induced Alterations in Brain eCB Levels

One of the more compelling cases for alcohol-eCB interactions regards a series of neuroimaging studies that used positron emission topography to examine CB_1 receptor binding in humans who smoke cannabis, and then separately in people with AUD.[56-58] Chronic cannabis use produced a striking pattern of CB_1 receptor downregulation in several (but not all) corticolimbic regions. The results were not surprising given that the psychotropic effects of cannabis are largely mediated by CB_1 receptor stimulation. Interestingly, patients with AUD showed a similar pattern of dysregulation, though were noted to exhibit decreased binding in all brain regions that were analyzed.[59,60] Moreover, the effects produced by chronic cannabis use returned to normal function after a protracted abstinence period, whereas the disruptions in patients with AUD persisted after 4 weeks of withdrawal from alcohol use. These findings suggest that CB_1 receptor downregulation is a common neuroadaptation to chronic substance use, although seemingly more extensive under alcohol exposure than with substances that directly interact with CB_1 receptors. This may suggest that alcohol has potent effects on the mechanisms of CB_1 receptor expression and function (e.g., signaling transduction, epigenetic changes). Alcohol is also a notable activator of neuroinflammation, which over the course of repeated use may temper the anti-inflammatory responses of exogenous/endogenous cannabinoid signaling.[61] Moreover, it is possible that alcohol may play a role in altering endogenous mediators of cannabinoid signaling (e.g., eCBs), from which lapses in the recovery of these signaling ligands influence the long-lasting deficits in CB_1 receptor signaling.

Substantial literature indicates that brain eCB content is altered by substances with abuse potential. In this regard, alcohol alters AEA and 2-AG content in the brain, and chronic alcohol exposure generally leads to impairments in eCB signaling mechanisms. Early in vitro studies demonstrated that chronic alcohol exposure increases both AEA and 2-AG formation in human neuroblastoma cells and primary cultures of rodent cerebellar granule neurons.[62-64] Subsequent studies have evaluated the effects of alcohol exposure on brain eCB levels and reported differential effects.[65] Currently, it is difficult to draw a firm consensus of these data given the plethora of responses induced by alcohol administration, which may include—in addition to sample preparation, brain-region specificity, and methodological differences—the differential mobilization of AEA and 2-AG. Highlighted below are some of these findings, summarized in Table 1.

Chronic alcohol exposure has been shown to increase AEA content in the limbic forebrain of rodents, whereas withdrawal decreased AEA in these brain regions.[66-69] This increase in AEA is consistent with the reduction in FAAH activity following chronic alcohol exposure.[66] By contrast, protracted (but not acute) withdrawal increased AEA content in the rat hippocampus.[70] Short-term alcohol exposure also has been reported to decrease AEA content in several brain regions including the amygdala, hypothalamus, and caudate putamen.[71] Regarding 2-AG, several studies describe both increases and decreases in striatal 2-AG content after chronic alcohol exposure.[67,68,72] Moreover, acute and protracted withdrawal from chronic intermittent alcohol exposure was observed to increase 2-AG content in the rat hippocampus.[70] In the PFC, acute alcohol exposure was associated with decreases in 2-AG content,[71] whereas voluntary consumption in genetically selected rats that were bred for high alcohol preference was shown to increase 2-AG in this region.[69] Drinking behavior in Sardinian alcohol-preferring (sP) rats also was associated with increases in striatal 2-AG content that were most evident during the acquisition and maintenance phases.[72] These varied responses between studies are likely influenced by methodological differences in the procedure employed to quantify eCB tissue content,[73] as well as by other experimental factors including the selection of rodent model, rat strain, duration and amount of alcohol exposure, and timepoints of withdrawal assessment. Emerging research also suggests the possibility of sex differences in alcohol-eCB interactions that may be specific to ovarian hormones.[69,74]

As opposed to bulk eCB tissue levels, some laboratories have utilized in vivo microdialysis approaches to estimate changes in eCB levels in flux.[73] These studies likewise have reported region-specific effects in alcohol administration, as well as the influence of several factors involved in the administration, dose, contingency, and prior history of alcohol exposure.[75,76] Seminal work from Larry Parsons' laboratory demonstrated that operant alcohol self-administration increased interstitial levels of 2-AG in the NAc without altering dialysate levels in the medial PFC.[77,78] Systemic administration of moderate doses of alcohol also increased 2-AG levels in a similar manner in alcohol-naïve rats, and this effect was potentiated in alcohol

Table 1. Summary of Alcohol-Induced Alterations in Brain eCB Levels

Type of Study (cell/species)	Alcohol Exposure	Effects	Brain Region
In vitro (human neuroblastoma cells)	Chronic alcohol	▲AEA	N/A
In vitro (rodent cerebellar granule neurons)	Chronic alcohol	▲AEA ▲2-AG	N/A
Ex vivo tissue content (male Swiss Webster mice)	Chronic vapor inhalation	▲AEA	Cortex
	Acute withdrawal	▼AEA	Cortex
Ex vivo tissue content (male Wistar rats)	Chronic liquid diet	▼AEA ▼2-AG	Midbrain
		▲AEA	Limbic forebrain
	Acute withdrawal	▼AEA	Limbic forebrain
Ex vivo tissue content (male Sprague-Dawley rats)	Acute withdrawal	►AEA ▲2-AG	Hippocampus
	Long-term withdrawal	▲AEA ▲2-AG	
	Short-term alcohol exposure (liquid diet for 24h)	▼AEA	Hypothalamus Amygdala Caudate putamen
		▼2-AG	PFC

Table 1. Summary of Alcohol-Induced Alterations in Brain eCB Levels (Continued)

Type of Study (cell/species)	Alcohol Exposure	Effects	Brain Region
Ex vivo tissue content (female and male alcohol-preferring AA rats)	Long-term alcohol consumption in female:		
	Before drinking session	▲AEA	PFC NAc CPu
		▲2-AG	CPu Amygdala Hippocampus
	After drinking session	▼AEA	PFC CPu Amygdala Hippocampus
		▲2-AG	PFC
	Long-term alcohol consumption in male:		
	Before drinking session	►AEA ►2-AG	PFC NAc CPu Amygdala Hippocampus
	After drinking session	▲AEA	NAc CPu
Ex vivo tissue content (male sP rats)	Long-term voluntary alcohol consumption	▲2-AG	Striatum
Ex vivo tissue content (male and female Wistar rats)	Acute withdrawal male	▼AEA ▼2-AG	BLA vmPFC
	Acute withdrawal female	▼AEA	vmPFC
In vivo microdialysis (male Wistar rats)	Alcohol self-administration	▲2-AG ►AEA ►2-AG	NAc mPFC
In vivo microdialysis (male Wistar rats)	Acute alcohol administration in naïve rats (low doses)	▲2-AG ▼AEA	NAc
	Acute alcohol administration in naïve rats (high doses)	▲AEA	
	Acute alcohol administration in alcohol-dependent rats	▲▲2-AG ► AEA	NAc
In vivo microdialysis (male Wistar rats)	Chronic alcohol exposure	▼2-AG ► AEA	CeA CeA / NAc

Note: ▲, increase;▼, decrease; ►, no effect; 2-AG, 2-arachidonylglycerol; AA rats, Alko alcohol rats; AEA, anandamide; BLA, basolateral amygdala; CeA, nucleus of the central amygdala; CPu, caudate putamen; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PFC, prefrontal cortex; sP rats, Sardinian alcohol-preferring rats; vmPFC, ventromedial prefrontal cortex.

dependence.[76] More recently, the authors observed that alcohol dependence resulted in the reduction of baseline 2-AG levels in the central nucleus of the amygdala (CeA), conferring a blunting of alcohol's mobilizing responses in this region.[79] Regarding AEA, alcohol self-administration did not differentially alter interstitial levels of AEA across several brain regions.[76,77,79] Interestingly, noncontingent alcohol administration reduced AEA in the NAc, whereas higher doses produced a milder increase in dialysate levels.[75,76,80] Alcohol dependence also did not appear to drastically alter baseline AEA levels in the CeA.[79]

Overall, it is clear that alcohol administration alters eCB responsivity, albeit in a manner that is dependent on several factors of exposure. What is less clear, however, is the manner in which alcohol may be mobilizing these responses, let alone with any given specificity to eCB signaling. Previous studies have shown that alcohol possesses cell membrane-disrupting properties that build tolerance over the course of repeated exposure. This resistance is conferred through the alteration of lipid membrane composition that includes changes in important glycerophospholipids such as phosphatidylinositol, cardiolipin, and several classes of amino glycerophospholipids (e.g., phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine).[81,82] The changes in phospholipid content vary with the nature of alcohol-induced perturbation, demonstrating higher depletion effects under intermittent versus continuous exposure conditions.[83] Acute withdrawal also has membrane-disordering consequences in different cellular compartments that were previously acclimated to the presence of alcohol.[81] Collectively, these findings suggest that alcohol exposure and withdrawal perturb the integrity of the cellular lipid bilayer, which may be important for determining the source of glycerophospholipid content available for eCB synthesis. In this regard, depletions in inositol phospholipid content would seemingly have profound implications in the ability to mobilize 2-AG synthesis relative to AEA systems that contain biosynthetic redundancies for recuperating losses.

The Influence of eCB Systems on Alcohol-Related Behaviors

Given the precedence for alcohol-eCB dysregulation, there are several avenues for which one might explore the role of eCB systems in addiction behavior. Although many studies point to the influence of CB_1 receptors, recent advancements have made it possible to discretely manipulate eCB signaling elements. Highlighted below are some of these investigations that underscore the involvement of eCB systems in alcohol-related behaviors. Table 2 provides a summary of the main findings for cannabinoid receptors.

CB_1 Receptors

The consensus of preclinical work demonstrates that activation of CB_1 receptors has a facilitatory effect on the motivation and consumption of alcohol. For example, systemic administration of the synthetic CB_1 receptor agonists WIN 55,212-2 and CP 55,940 both increased spontaneous drinking in sP rats and mice.[84-87] These synthetic agonists also increased operant responding for alcohol in Alko alcohol rats and Indiana P rats, as well as in non-selected Wistar rats.[88-90] The facilitatory effect on alcohol consumption likely involves the activation of mesolimbic CB_1 receptors, given that both systemic and intracranial infusions of WIN 55,212-2 into the posterior ventral tegmental area increased binge-like alcohol intake.[91] Additional studies have shown that WIN 55,212-2 administration increased the magnitude of excessive drinking elicited by the alcohol deprivation effect.[92,93] Conversely, the pharmacological blockade of CB_1 receptors by the CB_1 antagonist/inverse agonist SR141716A (rimonabant) decreased alcohol consumption in non-selected and alcohol-preferring rats and mice.[86,94-98] This decrease was observed in both dependent and non-dependent rodent models[98,99] and was further associated with reduced motivation for alcohol.[97,100] SR141716A also reduced the magnitude of alcohol deprivation effect responses in alcohol-preferring rats[72,90,101] and treatment with other selective CB_1 antagonists/inverse agonists recapitulated many of these same effects.[102-105] Consistent with this, the genetic ablation of CB_1 receptors in mice attenuated alcohol preference and intake,[86,106-108] diminished the influence of SR141716A pharmacology,[108] and reduced preference for environments previously paired with alcohol reward (e.g., conditioned place preference [CPP]).[109] This likely has some bearing with the modulation of mesolimbic dopamine given that alcohol's ability to increase NAc dopamine release was compromised in CB_1 receptor knockout mice.[106]

Overall, these findings demonstrate that while activation of CB_1 receptors promotes alcohol consumption, the pharmacological blockade or genetic deletion of these receptors has the opposite effect.[110] The results underscore the importance of CB_1 receptors in alcohol-related behaviors, although there is less clarity regarding the signaling substrates that mediate these responses. In this regard, the authors' recent work demonstrated that SR141716A infused directly into the NAc shell decreased alcohol self-administration and this tempering response was recapitulated with the exogenous administration of 2-AG, but not AEA into this region.[111] The findings suggest the possibility of 2-AG–CB_1 signaling being an important mediator in the reinforcing effects of alcohol, although the possibility of non-cannabinoid signaling pathways has not yet been ruled out. These findings have translational relevance in the clinic given that polymorphisms of the *Cnr1* gene that encodes for CB_1 receptors were associated with symptoms of AUD.[112]

CB_2 Receptors

Although numerous findings corroborate the involvement of CB_1 receptors in alcohol-related pathology, the possible role of CB_2 receptors remains somewhat controversial. Brain CB_2 signaling is typically engaged under marked conditions of neuroinflammation and tissue trauma,[113] and the extent to which drugs of abuse may elicit such phenotypes is currently under investigation. That being stated, sub-chronic treatment with the CB_2 receptor agonist JWH-015 was reported to increase chronic stress-induced alcohol consumption, whereas similar protocols with the CB_2 receptor antagonist AM630 prevented alcohol preference.[114] The naturally available full-agonist of CB_2 receptors, beta-caryophyllene, had dissimilar effects and instead decreased preference and consumption as well as inhibited the expression of alcohol-induced CPP.[115] Studies using the selective CB_2 agonist JWH-133 also reported contradictory findings, in some cases showing the attenuation of alcohol-induced CPP and operant self-administration,[116,117] and in others having no effect on these behaviors.[118,119] The varied responses may be due to experimental factors such as the method and duration of alcohol exposure, the mouse strain utilized, or the dose of agonist administered prior to testing.

The blockade of CB_2 receptors has somewhat more consistent effects that align with increased reinforcement and motivation for alcohol. For example, repeated administration of the antagonist AM630 increased operant alcohol self-administration in mice,[117] although others reported no effects on alcohol intake or alcohol-induced CPP.[114,118] Behavioral phenotyping in CB_2 receptor knockout mice has shown that these animals exhibit increased alcohol preference and consumption, elicit more physical signs of alcohol dependence,[120] and express higher alcohol-induced CPP than wild-type controls.[118,120] By contrast, knockout mice of a different strain did not exhibit significant differences in limited-access drinking,[118,121] but interestingly showed an increase in alcohol intake under forced alcohol exposure and group-housing conditions. These data suggest the possibility that CB_2 receptors may tie into complex interactions of alcohol and stress that is facilitated by the social environment.[121] Targeting the deletion of CB_2 receptors in dopamine neurons also reduced alcohol consumption and mitigated the expression of alcohol-induced CPP in DAT-*Cnr2* conditional knockout mice.[116] These findings may bear some translational relevance in the clinical field given that polymorphisms in the CB_2 receptor gene (*Cnr2*) were associated with AUD in Japanese populations.[114]

Inhibition of eCB Clearance

The modulation of cannabinoid receptors provides a strong basis for alcohol-eCB interactions; however, the recent development of novel pharmacological and genetic tools that prevent the clearance of eCBs provides a means to discern the roles of these lipids in alcohol-induced behavior. Table 3 summarizes the information below.

FAAH Inhibition

The inhibition or genetic deletion of the clearance enzyme FAAH results in an increase in AEA levels as well as other acylethanolamines such as oleoylethanolamine and palmitoylethanolamine.[122] Growing evidence suggests that impairment of FAAH may prime sensitivity to the reinforcing effects of alcohol and attenuate the negative consequences of excessive drinking. For example, acute administration of the FAAH inhibitor URB597 in mice increased alcohol preference and consumption, while also reducing sensitivity to the motor-impairing responses of intoxication.[85,123,124] Similar effects were observed in the genetic deletion of FAAH in mice,[85,123,124] that among other attributes promoted the quick recovery of alcohol-induced motor discoordination. The pharmacological effects of URB597 were further abrogated in CB_1 receptor and FAAH knockout mice, and behavioral sensitization to repeated alcohol administration was diminished in these mouse lines.[125] Contrary to the findings in mice, URB597 administration did not alter voluntary drinking in alcohol-preferring rats or operant responding in non-selected Wistar rats.[126,127] The authors observed similar findings with the administration of the selective FAAH inhibitor PF-3845 in both dependent and nondependent rats.[79] Thus, although FAAH inhibition may differentially alter alcohol-related behaviors in mice, it is less clear whether similar phenotypes exist in rat models. Alternatively, several studies have demonstrated that inhibiting FAAH more discretely within corticolimbic areas of the brain resulted in observable phenotypes. For example, the local administration of URB597 into the PFC of non-selected rats facilitated operant alcohol self-administration, and this effect was consistent with observations of decreased FAAH expression and activity in the PFC of alcohol-preferring Alko alcohol rats.[126] By contrast, infusions of URB597 into the CeA or the basolateral amygdala reduced alcohol self-administration in Marchigian Sardinian alcohol-preferring (msP) rats, while having no effect in non-selected Wistar rats.[128] The msP rat line has been previously shown to exhibit elevated FAAH activity in amygdalar brain regions,[129] suggesting that facilitation or inhibition of alcohol drinking may largely depend on the status of AEA signaling in these corticolimbic regions. Thus, peripheral administration of an FAAH inhibitor is likely to offset the region-specific differences in AEA clearance, not surprisingly culminating in a null response on alcohol drinking.

Recent work has explored the contribution of FAAH mechanisms in driving alcohol-seeking behavior. Consistent with the studies above, FAAH inhibition in mice reduced reinstatement-induced drinking in a CB_1-dependent manner.[130] In rats, the peripheral administration of URB597 did not facilitate operant responding in an alcohol reinstatement model,[127] nor did it moderate alcohol reinstatement driven by pharmacological stressors. However, the local administration of URB597 into the lateral habenula reduced voluntary consumption and preference in alcohol-dependent rats[131] and reduced alcohol-

seeking behavior; these effects were effectively reversed by co-administration of rimonabant. The lateral habenula has garnered recent interest in the addiction field given its role in mediating negative valence information that may contribute to the negative symptoms of withdrawal.[132] Dysregulation of FAAH is also observed in the clinic, given that a missense mutation in FAAH (e.g., the C385A polymorphism) was associated with heightened prevalence of AUD,[133,134] and increased risk of developing alcohol problems in young people.[135]

Inhibition of eCB Transport

Currently, the mechanisms mediating fatty acid sequestration and membrane transport of the eCBs are unclear, although a few studies have elucidated the effects of an active metabolite of acetaminophen (i.e., AM404) in modulating alcohol-related behaviors. AM404 is thought to prevent the uptake of AEA and 2-AG, in effect prolonging synaptic signaling of these lipids.[136-138] In mice, AM404 reduced alcohol-seeking behavior and consumption.[139] Similarly, this compound reduced alcohol self-administration in Wistar rats at doses that did not alter saccharin self-administration, though no effects were observed in cue- or stress-induced reinstatement models.[140]

MAGL Inhibition

Although many studies have characterized the role of AEA/FAAH signaling systems on alcohol-related behaviors, the possible relevance of 2-AG/MAGL is only beginning to be explored with the development of selective and efficacious tools for inhibiting MAGL. In this regard, the authors have shown that local administration of the selective MAGL inhibitor URB602

Table 2. Summary of CB Receptor Influence on Alcohol-Related Behaviors

CB Receptor Manipulation	Effects
CB_1 receptor agonists	▲spontaneous drinking in alcohol-preferring rodents ▲alcohol SA in rats ▲binge-like alcohol intake in mice ▲alcohol-seeking behavior
CB_1 receptor antagonists	
systemic administration	▼alcohol preference ▼alcohol consumption in rodents ▼alcohol-seeking-behavior
localized infusions: intra-NAc	▼alcohol SA
intra-VTA	▼alcohol SA
intra-mPFC	►alcohol SA in normal rats
intra-PFC	▼alcohol SA in alcohol-preferring rats
CB_1 receptor knockout mice	▼alcohol preference ▼alcohol consumption in rodents ▼CPP ▼alcohol-induced NAc dopamine
CB_2 receptor agonists	▲alcohol consumption in stressed mice ▼CPP / ►CPP ▼alcohol preference ▼alcohol consumption / ►alcohol consumption ▼alcohol SA
CB_2 receptor antagonists	▲alcohol SA
CB_2 receptor knockout mice	▲alcohol consumption ▲alcohol preference ▲physical signs of withdrawal ▲CPP

Note: ▲, increase; ▼, decrease; ►, no effect; CB, cannabinoid; CB_1 receptor, cannabinoid receptor type 1; CB_2 receptor, cannabinoid receptor type 2; CPP, conditioned place preference; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PFC, prefrontal cortex; SA, self-administration; VTA, ventral tegmental area.

Table 3. Summary of eCB Clearance Inhibition Influence on Alcohol-Related Behaviors

eCB Clearance Manipulation	Effects
FAAH inhibitors	
systemic administration	▲alcohol preference in mice, but not rats ▲alcohol consumption in mice, but not rats ▼sensitivity to alcohol intoxication
localized infusions: intra-PFC	▲alcohol SA in rats
intra-amygdala	▼alcohol SA in msP rats ►alcohol SA in Wistar rats
intra-LHb	▼alcohol preference in alcohol-dependent rats ▼alcohol consumption in alcohol-dependent rats ▼alcohol-seeking behavior
FAAH knockout mice	▲alcohol preference ▲alcohol consumption ▼sensitivity to alcohol intoxication
eCB transport inhibitor	▼alcohol seeking ▼alcohol consumption ▼alcohol SA in rats
MAGL inhibitors	
systemic administration	▼alcohol intake in alcohol-dependent rodents ►alcohol intake in non–alcohol-dependent rodents
localized infusions: intra-NAc shell	▼alcohol SA in rats
intra-LHb	▼alcohol consumption in alcohol-dependent rats ►alcohol consumption in non–alcohol-dependent rats

Note: ▲, increase; ▼, decrease; ►, no effect; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; LHb, lateral habenula; MAGL, monoacylglycerol lipase; msP rats, Marchigian Sardinian alcohol-preferring rats; NAc, nucleus accumbens; PFC, prefrontal cortex; SA, self-administration.

into the NAc shell reduces operant alcohol self-administration in rats.[111] In addition, acute administration of the inhibitor MJN110 reduced operant self-administration in alcohol-dependent rats, and in separate studies reduced voluntary drinking in dependent mice using the inhibitor JZL184.[79] Consistent with these findings, increased MAGL activity was observed in the lateral habenula of dependent rats, and intracranial infusions of JZL184 reduced alcohol consumption in a CB_1-dependent manner.[131] Thus, as opposed to the varied responses obtained with systemic FAAH inhibitors, the dysregulation of 2-AG/MAGL signaling in dependence appears to be a pervasive or stable phenotype. That stated, a more time-dependent profiling of the changes induced by chronic alcohol exposure and withdrawal is warranted and should provide a better means of discerning the therapeutic potential of FAAH and MAGL inhibitors in AUD.

Endocannabinoids and Withdrawal-Related Anxiety

Repeated cycles of alcohol intoxication and withdrawal induce neuroadaptations that alter the motivational mechanisms involved in compulsive alcohol seeking and drinking.[141] Although initial use is motivated by the hedonic effects of alcohol, prolonged exposure results in the blunting of brain reward pathways that are overcome pharmacologically by escalating alcohol intake. At the same time, opponent processes involved in the remediation of mood states gain traction and contribute to the expression of negative affect during periods of alcohol abstinence. This rise in sensitivity marks a transition point where alcohol use becomes an effective means of alleviating negative behavioral states, thus creating a psychological tangent for the

progression of AUD. Namely, withdrawal-induced increases in negative affective states (e.g., hyperkatifeia[4]) arise from the combination of stress signaling factors that activate areas of the extended amygdala (e.g., corticotropin-releasing factor [CRF]) and diminished performance of the mechanisms that constrain these responses through so-called "anti-stress" functions.[142] Growing evidence implicates the eCB system as a prevailing mechanism in the regulation of stress signaling,[112,143,144] and by extension of this basic function, reflects the loss of a critical "anti-stress" mechanism in AUD.[145] Highlighted below is some of the research supporting the framework for dysregulated eCB signaling in the manifest of negative affective behavior associated with alcohol withdrawal.

Substantial evidence shows that eCB systems play a key role in the modulation of stress signaling, wherein disruptions of eCB signaling can facilitate anxiety-like states.[146] CB_1 receptors are expressed in high or moderate densities across many regions involved in the expression of anxiety, including the CeA, basolateral amygdala, PFC, ventral hippocampus, and bed nucleus of the stria terminalis.[8,147,148] As with the findings observed in human subjects with AUD, the downregulation of CB_1 receptors appears to be an important attribute of mood affective disorders, at least within subcortical regions that are posited to interact more frequently with upstream hormonal regulators.[149]

Cannabis use in humans is known to alter anxiety-like states in a dose-dependent manner.[150,151] For example, the acute administration of Delta^9-tetrahydrocannabinol (THC) produces anxiolytic responses at low doses,[152-155] but elicits anxiogenic effects with progressively higher doses.[152,156,157] Synthetic agonists of CB_1 receptors display similar propensities in rodents that are abrogated with a CB_1 receptor antagonist.[158,159] Interestingly, not all agonists modulate anxiety-like behavior in the same manner and instead display complex interactions with the testing environment. Indeed, low doses of the agonist HU-210 were observed to contain anxiolytic-like effects in a model of defensive withdrawal behavior when tested in novel environments, whereas similar doses under habituated settings produced anxiogenic-like responses.[160] Given that CB_1 receptors are located on the terminals of glutamatergic and GABAergic neurons,[161] it is hypothesized that the regulation of anxiety-like behavior may relate more specifically to the subpopulation of neurons influenced by CB_1 receptor activation. In this regard, studies using conditional mutant mice lacking CB_1 receptors within specific neurons reported that low-dose activation of CB_1 receptors on glutamatergic neurons was associated with anxiolytic-like responses, whereas high doses of agonist that disrupted GABAergic signaling were anxiogenic.[162-164]

There is now considerable evidence demonstrating that elevations in eCB levels (via the inhibition of clearance mechanisms) modulate anxiety-like behavior without inducing the same biphasic responses obtained with CB_1 receptor agonists. For example, the indirect stimulation of AEA signaling by FAAH inhibitors reduced the expression of anxiety-like behaviors in rodents but did so specifically under stressful or aversive conditions.[129,165-168] Similar effects were obtained in FAAH knockout mice.[169,170] In addition to AEA/FAAH signaling, there is evidence supporting the role of 2-AG/MAGL in the regulation of anxiety-like behavior. In this regard, the MAGL inhibitor JZL184 produced anxiolytic-like effects in rodents mainly under heightened stress conditions (e.g., brightly lit environments, following restraint stress).[165,168,171-174] Unlike the anxiolytic effects of FAAH inhibitors that are strongly associated with CB_1 receptor signaling,[144] both CB_1 and CB_2 receptors have been implicated in the anxiety-reducing properties of MAGL inhibitors;[173-176] to date, however, the preponderance of evidence suggests a CB_1 receptor contingency.

The authors' recent work with msP rats provides collective evidence of the strong relation between dysregulated AEA/FAAH signaling and innate symptoms of anxiety.[129] In this regard, msP rats are genetically selected for increased alcohol preference and consumption, as well as for the heightened expression of anxiety-like behavior.[177] Accordingly, the authors observed that msP rats displayed a sensitized stress response in the CeA and provided evidence of diminished AEA neurotransmission driven by increased clearance of this lipid by FAAH. Inhibition of FAAH with PF-3845 rescued the msP phenotype in several models of anxiety-like behavior, likely by restoring the integrity of stress-gating control in the CeA. Subsequent work demonstrated that local administration of the inhibitor URB597 into the CeA reversed the anxiety-producing effects of restraint stress, whereas no effects were observed in non-selected Wistar rats.[128] Consistent with this, the authors also have examined the effects of FAAH and MAGL inhibitors on withdrawal-induced anxiety-like behaviors in rodents and found that both inhibitors were effective in reducing these responses.[79] Given the tempered effects of systemic FAAH inhibitors in alcohol drinking behavior, it is tempting to suggest that AEA and 2-AG may be regulating different components of the addiction process, the former being more attuned to the regulation of basal anxiety levels and the latter being consequential of alcohol-induced perturbations. How this may fit into a gain- or loss-of-function model that can inform the therapeutic relevance of eCB clearance inhibitors remains to be elucidated. Additionally, the interactive role of eCB systems with stress-inducing factors such as CRF and other stress-constraining mechanisms such as cortisol/corticosterone is not well understood. In this regard, previous work suggests that neuroadaptations involving CRF-driven stimulation of FAAH coincide with the depletion of AEA-mediated constraint of the amygdala,[129,178] whereas the delayed and blunted release of corticosterone in msP rats[179] may present a challenge in mounting 2-AG remediation.[180]

Unlike the selective FAAH or MAGL inhibitors, the increase of AEA and 2-AG levels with the dual eCB clearance inhibitor JZL195 has little effect on reducing anxiety-like behavior

and instead appears to have anxiogenic-like properties.[165,181] Recently, the authors observed evidence of an anxiolytic-like effect with high doses of JZL195 on the elevated plus maze, but similar treatments had no effect in the light/dark box assay.[168] Moreover, treatment with the MAGL inhibitor JZL184 in FAAH knockout mice, mimicking the putative inhibitor properties of JZL195, did not produce any effects on anxiety-like behaviors. It should be borne in mind that dual FAAH/MAGL inhibition produced cannabimimetic effects[182] and prolonged changes in 2-AG signaling (via MAGL inhibitor treatment in FAAH knockout mice) that were associated with cannabinoid receptor dysregulation, tolerance to antinociception, and increased sensitivity to rimonabant-precipitated withdrawal behavior.[183] The potential role of dual FAAH/MAGL inhibition has not been thoroughly examined in alcohol-dependent rodents, but has been shown to contain neurogenesis-suppressing effects in the dentate gyrus in the same manner as the combined treatment of acute alcohol with a CB_1 agonist.[184]

Other studies have observed that the loss of 2-AG signaling through the genetic or pharmacological inhibition of synthase mechanisms is associated with anxiogenic-like responses. For example, DAGL-alpha knockout mice exhibit increased anxiety-like behaviors relative to their wild-type littermates,[185,186] and these effects were reversed by the administration of JZL184.[185] In the same regard, the DAGL inhibitor DO34 produced anxiogenic-like effects,[187] although the extent to which prior stress conditions may differentially influence the expression of anxiety-like behavior remains to be elucidated. Given evidence of alcohol's mobilizing properties of 2-AG signaling, it is possible that DAGL inhibition may serve as a novel therapeutic for the treatment of AUD. Indeed, recent studies are providing insight into the possible therapeutic relevance of DAGL inhibition in reducing alcohol consumption without precipitating negative affective behaviors associated with chronic alcohol exposure and withdrawal.[188]

In addition to preclinical work, clinical studies are underway to evaluate the therapeutic efficacy of eCB enzyme inhibitor treatment in humans. Currently, there is more information on pharmacological inhibitors of FAAH given that selective inhibitors of MAGL have been characterized only recently.[189] The FAAH inhibitor PF-04457845 has entered Phase 2 clinical testing for the treatment or study of several conditions including chronic pain, fear response, Tourette's syndrome, and cannabis use disorder. PF-04457845 was found to be safe, well tolerated, and—although showing negligible effects for analgesia—successful in facilitating fear extinction behavior in healthy individuals.[190,191] More recently, PF-04457845 was reported to reduce withdrawal symptoms and cannabis use in patients with cannabis use disorder.[192] Other FAAH inhibitors, such as JNJ-42165279 and ASP3652, also were found to be safe and well tolerated; although confirming the lack of efficacy for chronic pain, these FAAH inhibitors displayed anxiolytic effects in people with social anxiety disorders.[193-197] By contrast, the FAAH inhibitor BIA 10-2474 caused widespread concern when high doses of this drug induced neurotoxic effects in healthy individuals, ending in the death of one volunteer.[198] It was later reported that BIA 10-2474 displayed substantial "off-targets" that were unique to this drug and likely responsible for inducing metabolic dysregulation and cellular death.[199] Although future studies should continue to ascertain the safety profile of FAAH inhibitors, the positive responses observed in people with cannabis use disorder bode well for substance abuse treatment. Together with the recent development of selective MAGL inhibitors (ABX-1431) in clinical trial testing,[200] serine hydrolase inhibitors represent a possible treatment avenue for restoring dysfunctional cannabinoid signaling in people with AUD.

Conclusion and Future Directions

Despite some inconsistencies in the literature, a preponderance of evidence suggests that alcohol exposure alters brain eCB signaling. Findings from the Parsons' laboratory demonstrated that acute alcohol self-administration elicits increases in eCB release that are tempered over repeated exposure;[76,79] however, readers are referred to the Alcohol-Induced Alterations in Brain eCB Levels section of this review for noteworthy distinctions. In addition, the method of alcohol exposure plays a marked role in the subsequent analysis of abstinence-related effects.[201,202] That stated, chronic alcohol exposure is generally associated with the disruption of eCB clearance mechanisms, impaired eCB-mediated forms of synaptic plasticity, and the downregulation of cannabinoid receptor function. The dysregulation of eCB signaling may be relevant given that eCBs play a prominent role in the maintenance of affective states and the constraint of stress responses, both of which serve as provocateurs of continued use and relapse. The remediation of eCB signaling remains an important goal for the possible treatment of AUD; however, this is unlikely to be achieved through the exogenous manipulation of CB_1 receptors that are fraught with concerns.[202-205] Accordingly, eCB clearance therapeutics may present an alternative pathway for restoring dysfunctional signaling elements, although further research is needed to better understand the consequence of eCB augmentation in dependence states across other relevant variables, including sex, brain regions, environment, emotional valence, pre-existing conditions, and neurohormones.[206]

Understanding of eCB signaling has greatly evolved since the discovery of eCBs nearly 30 years ago. This was fueled by technological advancements in the isolation, detection, and sequencing of the two primary eCBs, as well as the crystallization of biosynthetic enzymes and receptor systems that enable them. Cutting-edge technology continues to be an important driver in the field for the identification of novel molecular species and distinctions in eCB function. For example, mass spectrometry analysis can be broadly applied to investigate the brain lipidome, from which metabolic products of eCB

degradation are utilized by downstream signaling pathways (e.g., eicosanoids) to mediate neuroinflammation.[207] This is coupled closely to the advancements of novel pharmacological tools such as DO34 and the NAPE-PLD inhibitor LEI-401[208] that will allow us to manipulate AEA and 2-AG signaling with great precision and selectivity. Moreover, the spatiotemporal resolution of such changes is fundamental to the understanding of eCB function and may provide insight on the purpose of having multiple endogenous ligands of cannabinoid receptors. Although traditionally studied with in vivo microdialysis, the recent development of G-protein coupled receptor activation-based eCB sensors offers subsecond resolution kinetics and robust fluorescence-based detection in awake-behaving rodents.[209] Finally, the development of novel positron-emission topography tracers such as [^{11}C]MK-3168[210] and [^{18}F]T-401[211] will allow the direct assessment of FAAH and MAGL activity under a number of planned clinical studies, including in people with AUD. Taken all together, emerging research appears to be on the precipice of divulging new information about the eCB system. The combination of selective pharmacology and in vivo capture methods remains an important endeavor in this research for answering fundamental questions of eCB function, its relation to stress and anxiety, and its higher-order influence in complex psychopathologies such as AUD and addiction.

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NIAAA 50th ANNIVERSARY FESTSCHRIFT

Alcohol and the Adolescent Brain: What We've Learned and Where the Data Are Taking Us

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This article is part of a Festschrift commemorating the 50th anniversary of the National Institute on Alcohol Abuse and Alcoholism (NIAAA). Established in 1970, first as part of the National Institute of Mental Health and later as an independent institute of the National Institutes of Health, NIAAA today is the world's largest funding agency for alcohol research. In addition to its own intramural research program, NIAAA supports the entire spectrum of innovative basic, translational, and clinical research to advance the diagnosis, prevention, and treatment of alcohol use disorder and alcohol-related problems. To celebrate the anniversary, NIAAA hosted a 2-day symposium, "Alcohol Across the Lifespan: 50 Years of Evidence-Based Diagnosis, Prevention, and Treatment Research," devoted to key topics within the field of alcohol research. This article is based on Dr. Tapert's presentation at the event. NIAAA Director George F. Koob, Ph.D., serves as editor of the Festschrift.

KEYWORDS: alcohol; adolescence; binge drinking; neuroimaging; magnetic resonance imaging; neuropsychological tests; young adults; drinking behavior

The past 50 years of research supported by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) have resulted in an accumulation of invaluable data to address the multifaceted problems surrounding underage drinking. Youth use of alcohol remains a pervasive social and public health concern in the United States and a leading cause of disability and mortality during adolescence.[1,2] Alcohol use in adolescence has a distinct pattern from adult drinking, whereby adolescents may have fewer drinking occasions but consume relatively high levels per occasion, referred to as binge or heavy episodic drinking and defined as consuming four or more standard ethanol consumption units on an occasion for females and five or more for males.[3-5] Highly prevalent among youth in Western countries is an intermittent pattern of heavy alcohol consumption that typically is associated with social leisure occasions on weekend nights.[6] Moreover, adolescent alcohol use, along with smoking and illicit drug use, has undergone changes in prevalence and patterns in recent decades. For example, alcohol use peaked in the mid-1990s, with approximately 50% of 12th graders reporting past-month alcohol use, followed by a steady long-term decline to 30% in 2018. In 2020, the downward trend reversed course, with 34% of 12th graders reporting past-month alcohol use.[1] Recent reports indicate that prevalence estimates for 2021 will need to account for impacts of the COVID-19 global pandemic on underage substance use behavior and availability.[7]

High-risk alcohol consumption patterns and associated problems alone increase risk for adverse outcomes—such as motor vehicle accidents, high-risk sexual behaviors, other illicit substance use, and mental health challenges—for adolescents who drink. These risks are further compounded by the fact that adolescence is a period of crucial brain development and maturation.[8,9] Neuroimaging studies have provided clear evidence that the brain (a) continues to develop throughout adolescence and into adulthood, and (b) undergoes important structural and functional changes in synaptic plasticity and neural connectivity during adolescence.[10,11] These changes and the enormous plasticity of the teen brain make adolescence a time of both great risk and great opportunity.[11]

This article begins with an overview of typical adolescent brain development, followed by a summary of four key themes in the current understanding of alcohol and the adolescent brain: (1) predictors of underage drinking; (2) consequences of alcohol on adolescent brain structure and function; (3) moderating and confounding factors, including age of onset, sex disparities, family history, co-use of other substances, and mental health comorbidities; and (4) reversibility of and recovery from alcohol misuse. The article concludes with a discussion of where the data lead us to reach the next milestones in NIAAA-supported research.

Typical Adolescent Brain Development

The brain of an adolescent, much like teenage behavior, undergoes significant developmental changes. This neurodevelopment continues after adolescence, typically until around age 25.[12-15] The maturational processes in the brain occur in stages, with more basic functions (e.g., motor and sensory functions) maturing first and areas such as the lateral temporal and frontal lobes, which are responsible for higher cognitive function (e.g., decision-making, attention), developing later in adolescence.[13] The prefrontal cortex is one of the last brain regions to complete its maturation. Its rate of change does not plateau until the third decade of life, in concert with typical developmental trajectories of cognitive abilities, such as decision-making, attention, and cognitive control.[16-18] The late maturation of the prefrontal cortex has been linked to risky behavior during adolescence, particularly if the limbic subcortical system develops earlier.[16]

Executive functioning typically matures during this developmental stage,[19] coincident with gray matter reductions and white matter growth.[20,21] Functional magnetic resonance imaging (fMRI) studies of executive behaviors have demonstrated increasing prefrontal activity and better inhibitory control with adolescent age.[22] Challenges in executive functioning have been observed in adolescents with a family history of alcohol use disorder (AUD),[23] repeated childhood trauma experiences,[24] and poor sleep,[25] all of which also have been identified as risk for adolescent binge drinking and AUD.[17,26,27] Deficits in control circuitry have been linked to impulsivity, sensation seeking, and alcohol use into early adulthood.[28]

One of the studies investigating adolescent alcohol use and its effects is coordinated by the National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA), which is conducting a multisite longitudinal study supported by funding from NIAAA and other National Institutes of Health partner institutes. Launched in 2012, this five-site consortium recruited a community cohort of 831 diverse adolescents ages 12 to 21 from five U.S. regions (Durham, North Carolina; Palo Alto, California; Pittsburgh, Pennsylvania; Portland, Oregon; and San Diego, California). Half the sample was enriched for key characteristics conveying risk for heavy drinking among adolescents (i.e., family history of substance use disorder, youth externalizing or internalizing symptoms, and having tried alcohol by age 14). Most of the sample (85%) reported very limited alcohol use at project entry; the remaining 15% exceeded typical age thresholds for alcohol at project entry in this cohort-sequential design.[29] At project entry and annually thereafter, participants received neuroimaging (high-

resolution structural, diffusion, and resting-state fMRI scans), neurocognitive testing, detailed substance use and mental health interviews; provided urine samples for drug testing as well as saliva samples for genetics and pubertal hormone assays; and completed various self- and parent reports on personality, behaviors, and environment.[29] NCANDA will continue to examine the interactive effects of typical development as well as adolescent alcohol use and executive dysfunction into early adulthood.

Resting-state fMRI findings from NCANDA and other studies have shown that intrinsic functional networks subserving cognitive control and limbic circuitry develop across adolescence and may be influenced by adolescent heavy drinking.[24,30,31] Moreover, the adverse effects of alcohol may be more prominent in girls than in boys.[32]

Predictors of Underage Drinking

Being able to identify youth at higher risk for alcohol misuse could lead to early intervention and ultimately help reduce the significant personal and public health burden of AUD; however, relatively few studies have explored individual-level precursors of adolescent alcohol use. Prospective longitudinal studies of substance-naïve youth are uniquely positioned to identify factors predating the onset of alcohol use. Squeglia et al. identified several markers of alcohol initiation by age 18 in 137 adolescents.[27] These markers included demographic and behavioral factors (e.g., male sex, higher socioeconomic status, early dating, more externalizing behaviors, positive alcohol expectancies), lower executive functioning, thinner cortices, and less brain activation in diffusely distributed brain regions.

NCANDA seeks to expand on these findings using a greater number of measurements in a large sample to lead to more accurate individual-level forecasting. The consortium is employing machine learning models, which can avoid multiple-comparison correction and reduce measures to a single, individual-level prediction.[33,34] NCANDA developed a model that distinguished youth who drink heavily from those who drink little or no alcohol, based on patterns of macrostructural and microstructural imaging metrics in multiple brain regions.[35] The analyses suggested delayed development of white matter connectivity among the older youth in the sample who drank heavily, as well as increased risk of subsequent heavy drinking in youth with more externalizing symptoms. These findings fit closely with those from the IMAGEN Consortium, which found that variability in personality, cognition, life events, neural functioning, and drinking behavior features predicted Alcohol Use Disorders Identification Test scores at ages 14 and 16.[36]

Neural Consequences of Underage Heavy Drinking

Gray Matter Volume

Unlike white matter, gray matter volume peaks in the primary school-age years, around age 10.[11] Squeglia et al. reported that youth who drank heavily (n = 75) (defined using modified Cahalan quantity x frequency criteria[37,38]) showed accelerated reductions in gray matter volumes in cortical lateral frontal and temporal areas compared to those who drank no or little alcohol (n = 59).[39] These results were largely unchanged with co-use of marijuana and other drugs; also, similar patterns of developmental trajectory abnormalities existed in males and females. This finding was replicated in the NCANDA cohort, which examined the influence of alcohol use on gray matter structure in 483 adolescents ages 12 to 21 both before and 1 to 2 years after the onset of heavy drinking.[13] For youth with no or low alcohol consumption, gray matter volumes declined throughout adolescence, with rates slowing in many brain regions in later adolescence. However, youth who initiated heavy drinking exhibited a steeper decline in frontal gray matter volumes. For both youth with no or low alcohol consumption and those with heavy drinking, cannabis use did not influence gray matter volume trajectories.

These findings were confirmed in a recent analysis spanning five time points in the NCANDA study and using linear mixed-effects models.[40] A greater number of past-year binge drinking episodes was linked to greater decreases in gray matter volumes in 26 of 34 bilateral Desikan-Killiany cortical parcellations tested. The strongest effects were noted in frontal regions as well as among younger adolescents; moreover, the effects largely attenuated in later adolescence. The gray matter volumes decreased most for individuals with greater numbers of binge-drinking episodes and recent binge drinking. These findings provide yet more evidence that adolescent binge drinking is linked to a greater risk of more prominent gray matter reductions during adolescence.[40]

Functional MRI studies further suggested that adolescents with histories of heavy drinking showed aberrant patterns of activation in response to cognitively challenging tasks,[41,42] including tasks of working memory and inhibition. In adolescents with a history of 1 to 2 years of heavy drinking, the aberrant activation was not linked to detectable deficiencies in task performance. However, if heavy drinking persisted longer, reduced task performance was often evident in the adolescents.[43,44] This pattern of results suggested that the brain may be able to compensate for subtle neuronal insults for a period of time, but if drinking patterns persist and become heavier, the brain may no longer be able to compensate and may be vulnerable to the effects of repeated and sustained heavy doses of alcohol.

White Matter Volume and Integrity

Throughout adolescence, white matter volume increases and matures, resulting in myelination that increases speed of neuronal transmission and modulates the timing and synchrony of neuronal firing patterns that convey meaning in the brain.[11] Squeglia et al. reported that adolescents who drank heavily showed attenuated white matter growth of the corpus callosum and pons relative to adolescents who did not drink.[39] Pfefferbaum et al. indicated that among those in the NCANDA sample who consumed no or little alcohol, white matter regions grew at faster rates in younger age groups and slowed toward young adulthood.[13]

To examine the potential for a neurotoxic effect of alcohol use on adolescent development of white matter, Zhao et al. conducted a whole-brain analysis of fractional anisotropy of NCANDA participants ages 12 to 21 at baseline.[45] For 63 adolescents who initiated heavy drinking, the researchers examined white matter quality before and after drinking onset and compared it to the white matter maturation trajectory of 291 adolescents with no or low alcohol consumption. Results showed deterioration of white matter integrity in youth who drank heavily compared with age- and sex-matched controls. Moreover, the slope of this reduction over time corresponded with days of drinking since the study entry.[45] Within-subject analyses contrasted developmental trajectories of youth before and after they initiated heavy drinking. These analyses suggested that drinking onset was associated with, and appeared to precede, disrupted white matter integrity. This disruption was greater in younger adolescents than in older adolescents, and was most pronounced in the genu and body of the corpus callosum.[45] It is possible that these brain structure changes may occur concomitantly with modifications in certain neurotransmitter and hormone secretion systems, which markedly influence the refinement of certain brain areas and neural circuits.[46]

Neurocognition

Along with altered development and maturation of gray and white matter, studies have reported neurocognitive consequences of underage drinking, such as impairments in attention,[47] verbal learning,[48,49] visuospatial processing,[47,50] and memory.[49] Neurocognitive deficits linked to moderate to heavy drinking during this critical developmental period may lead to direct and indirect changes in neuromaturational course, with effects that may extend into adulthood. Squeglia et al. examined neurocognitive function in adolescents who drank heavily, moderately, or not at all, based on the Cahalan classification system.[51] Their findings suggested that initiation of moderate to heavy alcohol use and incurring hangovers during adolescence may adversely influence neurocognitive functioning. For females, more drinking days in the past year predicted a greater reduction in performance on visuospatial tasks, in particular visuospatial memory, from baseline to follow-up. For males, a tendency was seen for more hangover symptoms in the year before follow-up testing to predict a relative worsening of sustained attention.[51]

Alcohol Cue Reactivity

Another set of studies demonstrated that youths who drank heavily exhibited greater brain activation while viewing alcohol advertisements[25,52-54] than while viewing ads for nonalcoholic beverages.[52] Adolescents are exposed to alcohol advertising materials on a daily basis in many countries. As studies in adults with AUD have shown atypical responses to alcohol-related materials,[55] Tapert and colleagues used fMRI analyses to determine whether similar response patterns existed in adolescents who drink.[52] The study included 15 adolescents ages 14 to 17 with AUD and 15 demographically similar adolescents who drank infrequently. The participants were shown pictures of alcoholic and nonalcoholic beverage advertisements during neuroimaging. Adolescents with histories of heavy drinking showed greatly enhanced neural activation while viewing the pictures of alcoholic beverages compared with pictures of nonalcoholic beverages. The extent of alcohol-related activation was greatest for those with the highest levels of monthly alcohol intake (see Figure 1). In contrast, youth with limited drinking histories showed similar levels of activation while viewing the two beverage picture types. These results demonstrated pronounced alcohol cue reactivity in heavy drinking teens, particularly in reaction to alcohol advertising materials.

Factors Contributing to Adolescent Alcohol Use

Age of Onset

Studies examining longer-term impacts of adolescent alcohol misuse have yielded mixed results. Some studies reported a maturing-out without significant consequences in adulthood, while others found ongoing effects on mental health, physical health, and social functioning, as well as higher levels of alcohol use and AUD.[56] Analyses using data from the National Longitudinal Alcohol Epidemiologic Survey determined that 40% of those initiating alcohol use before age 15 were diagnosed with AUD at some point in their lives compared to only 10% of those who delayed the onset of drinking until age 21 or later.[57]

The first study of adolescents (ages 12 to 15 at baseline; $N = 215$) to assess the association between age of adolescent drinking onset and neurocognitive performance found that earlier age of drinking onset predicted poorer performance on tasks requiring psychomotor speed and visual attention. Similarly, an earlier age of onset of regular (weekly) drinking predicted poorer performances on tests of cognitive inhibition and working memory.[58] This study suggested that early onset

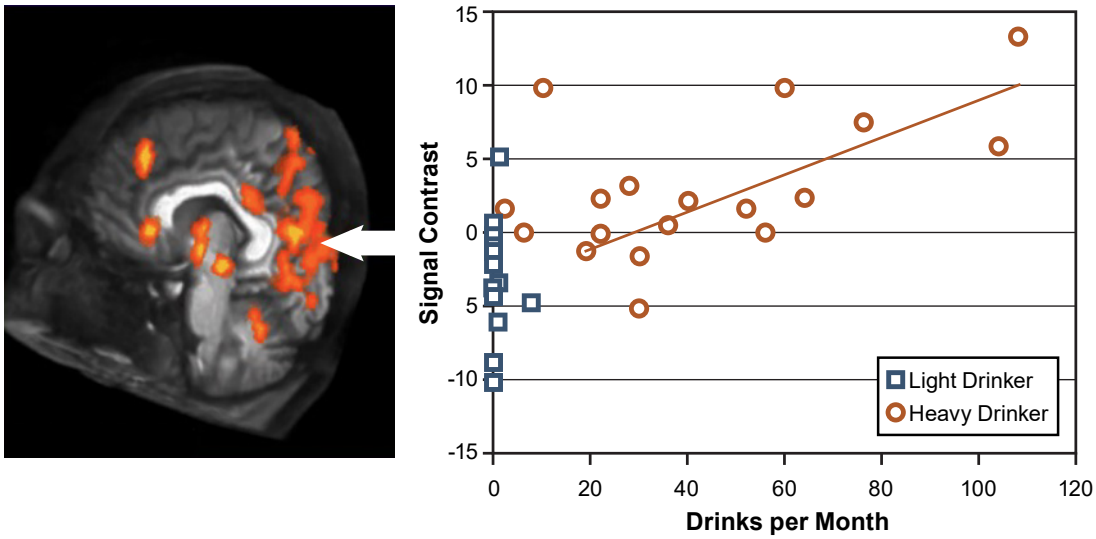

Figure 1. Response to alcohol pictures in youth with heavy versus light drinking. Brains of youths who drank heavily activated strongly in response to seeing alcohol advertisements but showed little brain response to nonalcoholic beverage ads; this difference (i.e., signal contrast) was smaller in youth who drank lightly. The difference in brain response was greatest in adolescents with the highest consumption levels and was especially strong in the left hemisphere (positive affect), limbic, and visual cortex areas. *Source:* Tapert et al., 2003.[52]

of drinking increased risk for subsequent neuropsychological dysfunction.

Sex Disparities

Several studies have reported that the associations between alcohol and brain structure and function differ by sex, especially in adolescents engaging in binge drinking. While not conclusive across the literature, female adolescents who engaged in binge drinking appeared to show effects such as blunted activation in frontal, temporal, and cerebellar cortices compared to females who did not drink, whereas male adolescents who engaged in binge drinking showed the opposite activation pattern.[59] Female adolescents ages 15 to 17 meeting criteria for AUD showed larger prefrontal cortex volumes than female controls, while male adolescents with AUD had smaller prefrontal cortex volumes than male controls.[60] A similar finding was observed for white matter.

Family History of AUD

Having a family member with AUD is associated with almost double the risk of initiating drinking in early adolescence.[57] Using fMRI, Spadoni et al. observed greater neural activity during rest and reduced activity during an active baseline condition were linked to denser family history of AUD.[61]

Mental Health Comorbidities

Adolescence is the peak time for both onset of substance misuse and emergence of mental illness, including anxiety disorders, bipolar disorder, major depression, eating disorders, and psychosis.[10] The National Survey on Drug Use and Health (NSDUH) estimated that 20% of adolescents had a mental illness that persisted into adulthood.[2] Moreover, adolescents with a past-year major depressive episode were more likely to be current binge alcohol users (6% vs. 4%).[2] However, it remains unclear how comorbid mental health problems contribute to and exacerbate the neurobiological effects of alcohol misuse.[4] Frontal and temporal cortical thinning may predict increased vulnerability to development of adolescent depression. In the NCANDA sample of 692 adolescents without a history of depression, the 101 youth who transitioned into depression were found at study baseline to have thinner cortices in the superior frontal cortex, precentral and postcentral regions, and superior temporal cortex, beyond effects attributable to age and sex.[62,63]

Adverse Childhood Events

Childhood trauma and post-traumatic stress symptoms have been shown to confer increased risk for adolescent and adulthood AUD, mental illness, and physical health problems.[64,65] Youth with trauma exposure showed thinner frontal cortices, and those with chronic post-traumatic stress disorder (PTSD) had smaller orbital frontal cortices[66] and less superior posterior cortical and cerebellar gray matter volume.[67] These observations indicate that trauma may be associated with structural brain aberrations.

NCANDA has also examined the relationship between childhood trauma and subsequent adolescent alcohol use.[68] In a sample of 392 NCANDA participants, adverse childhood event history was linked to greater self-reported executive dysfunction spanning four annual follow-ups. Greater childhood trauma also was linked to less connectivity in sensorimotor and cognitive control networks (i.e., the bilateral dorsal anterior cingulate cortex, right anterior insula, right intraparietal sulcus,

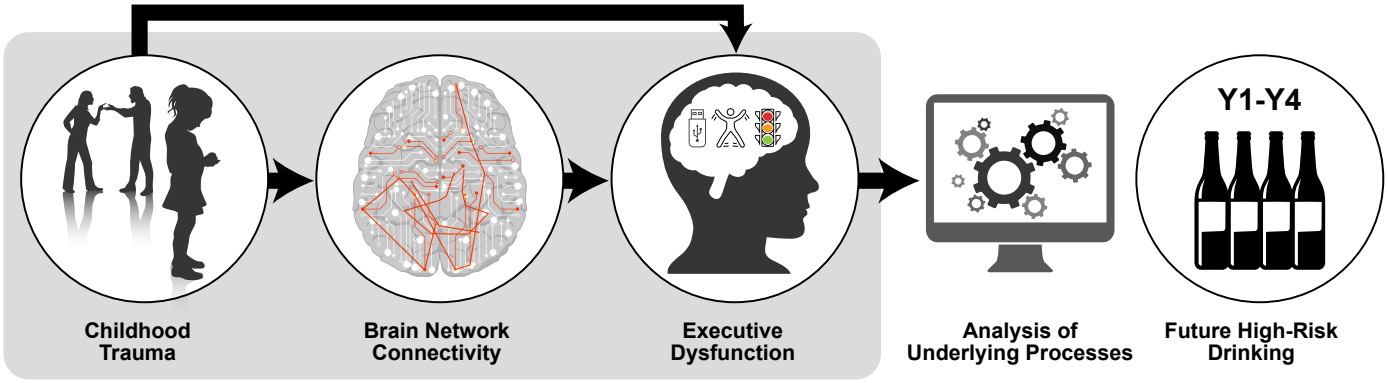

Figure 2. Model depicting how childhood trauma may lead to subsequent high-risk drinking. *Note:* Y1-Y4, Year 1 through Year 4. *Source:* Silveira et al., 2020.[24]

and bilateral pre- and postcentral gyri hub regions) at baseline. This reduced connectivity explained the relationship between executive dyscontrol and subsequent increased frequency of adolescent binge drinking (see Figure 2).[24]

Poor Sleep

Sleep patterns change substantially during adolescence and emerging adulthood.[69] Lack of sleep, going to sleep relatively late, and large weekend-weekday sleep differences all are risk factors for alcohol use in adolescents and young adults.[70] Similarly, in the NCANDA sample, sleep difficulties in adolescence predicted later substance use problems.[71] The reverse has also been seen, with acute and chronic alcohol intake altering sleep structure and electroencephalography patterns[72] in older adolescents[73] and adults.[69] NCANDA will continue to longitudinally examine whether these changes remain evident into adulthood and how alcohol use influences sleep neurobiology.

Use of Other Substances

Co-use of multiple substances may influence the relationship between alcohol use and neural integrity. For example, during a spatial working memory task, adolescents with co-occurring AUD and cannabis use disorder showed less inferior frontal and temporal neural activation but a greater medial frontal response compared to adolescents with AUD alone.[74] Co-use of alcohol with cannabis also may adversely influence executive functioning.[75] Given the high rates of co-occurring alcohol and other substance use during adolescence,[76] future well-powered studies will benefit from detailed analyses of various combinations of substances of abuse on neural and neurocognitive outcomes.

Recovery From Consequences of Adolescent Heavy Drinking

In adults with AUD, improvements in attention and concentration, reaction time, and memory are generally seen after 2 to 8 weeks of abstinence;[77] however, executive functioning, processing speed, visuospatial, and verbal skills appear more resistant to recovery,[78] and spatial processing deficits may persist for years.[79] Younger adults tend to recover more quickly and completely than older adults (i.e., over age 50).[80] As mentioned previously, preliminary evidence suggested that adolescent heavy drinkers showed greater response to alcohol cues,[54] more emotional reactivity and poorer distress tolerance,[81] and poorer visuospatial performance compared with adults.[82] These effects remitted after a month of abstinence, indicating that some deficits are linked to alcohol intake and may be transitory. However, executive dysfunction[81] and negative mood states[83] did not remit within 4 weeks of abstinence, suggesting that these differences may have predated the onset of heavy drinking or may take more time to recover. As reported by Infante et al., cortical gray matter volume decreases were greater in proximity to reported drinking episodes in a dose-response manner, suggesting a causal effect and raising the possibility that normal growth trajectories may recover with alcohol abstinence.[40] However, other studies have suggested that impaired visuospatial functioning following adolescent AUD persisted even after reduced levels of use.[84]

Where Do the Data Lead Next?

Longitudinal studies with large, diverse, representative samples of youth and a range of detailed measures are key to helping understand the behaviors that convey disadvantages to adolescent and young adult development and outcomes. To date, a handful of large-scale multisite studies are being conducted to

gain insight into the consequences of adolescents transitioning into and out of substance use. These include the largest long-term study of brain development in the United States, the Adolescent Brain Cognitive Development (ABCD) Study, which is currently underway; NCANDA; the IMAGEN study in Europe; the Pediatric Imaging, Neurocognition, and Genetics (PING) study; and the Lifespan Human Connectome Project (HCP) study. NCANDA has already been able to confirm impressions from prior smaller studies that adolescent heavy drinking appears linked to accelerated gray matter decline,[40] disrupted functional connectivity,[30] and reduced cognitive performance. Determining the degree to which these effects remit or persist with alcohol abstinence or reduced use will be a key next step in this line of work.

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The Synaptic Interactions of Alcohol and the Endogenous Cannabinoid System

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PURPOSE: A growing body of evidence has implicated the endocannabinoid (eCB) system in the acute, chronic, and withdrawal effects of alcohol/ethanol on synaptic function. These eCB-mediated synaptic effects may contribute to the development of alcohol use disorder (AUD). Alcohol exposure causes neurobiological alterations similar to those elicited by chronic cannabinoid (CB) exposure. Like alcohol, cannabinoids alter many central processes, such as cognition, locomotion, synaptic transmission, and neurotransmitter release. There is a strong need to elucidate the effects of ethanol on the eCB system in different brain regions to understand the role of eCB signaling in AUD.
SEARCH METHODS: For the scope of this review, preclinical studies were identified through queries of the PubMed database.
SEARCH RESULTS: This search yielded 459 articles. Clinical studies and papers irrelevant to the topic of this review were excluded.
DISCUSSION AND CONCLUSIONS: The endocannabinoid system includes, but is not limited to, cannabinoid receptors 1 (CB_1), among the most abundantly expressed neuronal receptors in the brain; cannabinoid receptors 2 (CB_2); and endogenously formed CB_1 ligands, including arachidonoylethanolamide (AEA; anandamide), and 2-arachidonoylglycerol (2-AG). The development of specific CB_1 agonists, such as WIN 55,212-2 (WIN), and antagonists, such as SR 141716A (rimonabant), provide powerful pharmacological tools for eCB research. Alcohol exposure has brain region–specific effects on the eCB system, including altering the synthesis of endocannabinoids (e.g., AEA, 2-AG), the synthesis of their precursors, and the density and coupling efficacy of CB_1. These alcohol-induced alterations of the eCB system have subsequent effects on synaptic function including neuronal excitability and postsynaptic conductance. This review will provide a comprehensive evaluation of the current literature on the synaptic interactions of alcohol exposure and eCB signaling systems, with an emphasis on molecular and physiological synaptic effects of alcohol on the eCB system. A limited volume of studies has focused on the underlying interactions of alcohol and the eCB system at the synaptic level in the brain. Thus, the data on synaptic interactions are sparse, and future research addressing these interactions is much needed.

KEYWORDS: endocannabinoid; alcohol use disorder; alcohol; synaptic; cannabis use disorder; cannabinoid receptor; cannabis; neurobiology

Alcohol use disorder (AUD) is a chronic, relapsing brain disorder, characterized by a compromised ability to control alcohol use despite adverse occupational, social, or health consequences. Results from a 2019 National Survey on Drug Use and Health found that 5% of individuals over age 12 had AUD, affecting 14.5 million people in the United States. Alcohol and cannabis products are a common polydrug combination.[1] Use of cannabinoids and alcohol alters many central processes, such as cognition, locomotion, and neuropeptide signaling.[2] Cannabis use is associated with the development and maintenance of AUD,[3] and individuals with cannabis use disorder (CUD) have an increased likelihood for development of comorbid AUD and double the risk for long-term problem drinking.[3] The risks associated with polysubstance use with alcohol and cannabis are greater than those associated with use of either drug alone.[3] Decriminalization has increased the availability and use of cannabis products[4] and polysubstance use, raising multiple social and health concerns.[5,6]

The high prevalence of comorbid AUD and CUD may be explained, in part, through findings indicating that alcohol and cannabis serve as a substitute for one another, as both have overall depressing effects on the central nervous system (CNS) and produce feelings of intoxication and euphoria.[7-9] Additionally, chronic ethanol administration in animal models causes neurobiological alterations similar to those elicited by chronic cannabinoid exposure,[10] and shared physiological and biochemical mechanisms may contribute to their combined use. Although cannabis and alcohol have varying targets and effects, both have been shown to interact through the endogenous cannabinoid (endocannabinoid [eCB]) system.[11] Ethanol changes the eCB system by altering the synthesis of eCBs, the synthesis of their precursors, and the density and coupling efficacy of cannabinoid receptor 1 (CB_1), a G protein–coupled receptor and a major receptor of the eCB system.[12-14] Furthermore, eCBs acting at CB_1 can modulate alcohol consumption in rats by affecting the activity of brain reward systems[15-17] and the function of the eCB system in AUD.[18-20]

Few studies have combined these two lines of research to fully understand the neurobiological substrates and synaptic interactions of alcohol and eCBs, or the therapeutic potential of targeting the eCB system for treating AUD. Therefore, this review provides an overview of the literature concerning how alcohol administration dysregulates eCB signaling and modulates eCB-mediated synaptic function. An emphasis is given to brain regions highly implicated in AUD and existing pharmacotherapies that target the eCB system and influence alcohol-perturbed synaptic functions. Additionally, a discussion of suggested future directions is provided to assist in addressing the lack of insights on the mechanisms and specific circuits at work in the synaptic interactions between alcohol and the eCB system.

The current literature indicates an urgent need for mechanistic studies to shed light on how perturbation of the brain eCB system contributes to development of AUD.

Method

For the scope of this review, preclinical studies were identified through queries of the PubMed database. The initial PubMed searches were undertaken in March 2021, with a final updated search date of June 2021, using the following terms: (endocannabinoids OR cannabinoid OR CB1 OR CB2 OR anandamide OR 2-arachidonoylglycerol OR FAAH OR MAGL OR DAGL OR NAPE-PLD) AND (chronic OR acute OR alcohol OR ethanol OR withdrawal) AND (hippocampus OR amygdala OR nucleus accumbens OR ventral tegmental area OR striatum OR cerebellum OR cortex OR prefrontal cortex) AND (synaptic OR synapse). This search yielded 459 articles. All articles containing relevant information and supporting the topics discussed in this review were included. These articles include research and findings related to the endocannabinoid pathway and acute, chronic, and withdrawal alcohol interactions in all brain regions and in specific regard to interactions pertaining to synaptic structure, function, and adaptations. Articles were excluded if they pertained only to clinical research, behavioral research, or findings outside of the brain and unrelated to synaptic/neuronal function. To support the topics covered, this review includes additional citations that did not appear in the search but that were considered relevant.

Results

The Endogenous Cannabinoid System: An Overview

The cannabinoid receptors were identified in the late 1980s, 2 decades after the discovery of the bioactive and psychoactive effects of delta-9-tetrahydrocannabinol (THC).[21,22] THC is one of 500 different compounds found in the plant *Cannabis sativa*, 85 of which are known cannabinoids (CBs).[23] THC is the compound mainly responsible for the psychotropic effects of cannabis and elicits its psychoactive effects through binding specific G protein–coupled receptors (GPCRs), termed cannabinoid receptors.[21,22] Two types of cannabinoid receptors were discovered via molecular cloning, the cannabinoid receptor type 1 (CB_1)[24] and the cannabinoid receptor type 2 (CB_2).[25-27] CB_1 is the most abundant GPCR in the mammalian brain, where it is primarily found on presynaptic terminals. CB_1 is also expressed at lower, but physiologically relevant, levels in most peripheral tissues.[20,28] CB_2 is abundant in the peripheral systems, and predominantly expressed in cells of the immune and hematopoietic systems. CB_2 is also present in the CNS, but at much lower concentrations compared to CB_1.[25,26,29,30] Discovering the role of CB_2 in the CNS is still ongoing.[26,31] Both CB_1 and CB_2 are primarily positively coupled to G_i/G_o proteins, and generally signal through inhibition of adenylate cyclase

(AC), inhibition of calcium channels, and activation of potassium channels, thus regulating numerous cellular processes.[19,20,28,32]

The discovery of these specific CB receptors led to the isolation of their endogenously formed ligands, including two lipid-derived principal eCBs, arachidonoylethanolamide (anandamide [AEA]) and 2-arachidonoylglycerol (2-AG).[33-36] AEA is a partial agonist with high affinity for CB_1, whereas 2-AG is a full agonist with a lower affinity for CB_1.[37] Other GPCRs and other targets also recognize CBs and related endogenous lipids; however, their role is less well understood.[38,39] For instance, both AEA and 2-AG bind to and activate the postsynaptic transient receptor potential vanilloid 1 and are agonists for several subtypes of the peroxisome proliferator-activated receptor family.[40] AEA and 2-AG are synthesized on demand from membrane phospholipid precursors. These eCBs are arachidonic acid derivatives, biosynthesized through a combination of several pathways.[19,41] AEA is mainly synthesized by the enzyme *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD),[42] but other enzymes important for synthesis include glycerophosphodiester phosphodiesterase 1 (GDE1), abhydrolase domain containing 4 (ABHD4) and the protein tyrosine phosphatase, non-receptor type 22 (PTPN22).[19,41] AEA is primarily catabolized by fatty acid amide hydrolase (FAAH), a serine hydrolase,[43] and 2-AG is synthesized from diacylglycerol (DAG) through the catalytic activity of diacylglycerol lipase alpha (DAGL-alpha) and DAGL-beta.[29,44] Catabolism of 2-AG occurs primarily by monoacylglycerol lipase (MAGL),[45] but other relevant contributors include abhydrolase domain containing 6 and 12 (ABHD6 and ABHD12).[46]

The eCB system is essential to many cellular processes and is implicated in signaling cascades that modulate synaptic processes such as calcium signaling, synaptic transmission, and neurotransmitter release.[19,28,41] In neurons, eCBs are synthesized and released postsynaptically, on demand, and in response to synaptic activity/membrane depolarization through calcium-dependent processes. The eCBs signal in a retrograde manner by traversing the synapse to bind their targets (i.e., CB_1) on the presynaptic membrane.

The eCBs activate CB_1 on both gamma-aminobutyric acid-ergic (GABAergic)[47-49] and glutamatergic terminals.[50] This presynaptic CB_1 activation provides feedback inhibition via the suppression of neurotransmitter release[51,52] in both inhibitory[53-55] and excitatory synapses.[56] However, alternative mechanisms for eCB release and CB_1 activation do occur; for example, the activity of metabotropic glutamate receptor subtype 5 (mGluR5)[57] and *N*-methyl-D-aspartate (NMDA) receptors[58,59] can stimulate eCB production and subsequent release to bind and activate presynaptic CB_1 receptors.[60-64] The eCB system therefore serves as a critical mechanism for modulating neuronal activity. CB_1 activation can lead to short- and long-term forms of plasticity, such as depolarization-induced suppression of inhibition/excitation and a form of synaptic long-term depression.[65,66] Long-term depression is characterized by a reduction in the efficacy of synapses in an activity-dependent manner.[65,66] The induction of these different forms of plasticity is probably linked to the activation of postsynaptic neurons that modulate concentration of eCBs at the synapse, the timing of CB_1 activation, and downstream effectors.[67] CB_2 is involved in a long-lasting cell-type–specific form of plasticity that triggers neuronal hyperpolarization.[68] The eCB system functions are reviewed by Lu and Anderson,[29] Basavarajappa,[32] and Basavarajappa et al.[41] Figure 1 provides a summary schematic of synaptic eCB signaling.

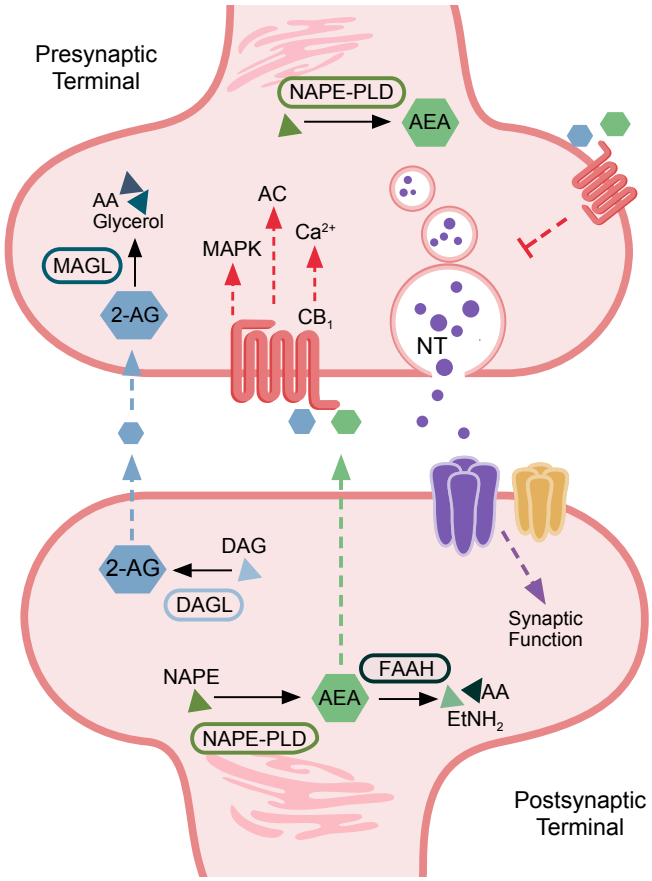

Figure 1. Summary schematic of endocannabinoid signaling in the synapse. A simplified description of the subcellular distribution of components of the endocannabinoid pathway is shown. Components include the major enzymes involved in regulating endocannabinoid levels (fatty acid amide hydrolase [FAAH], *N*-acyl phosphatidylethanolamine [NAPE], NAPE-specific phospholipase D [NAPE-PLD], monoacylglycerol lipase [MAGL], and diacylglycerol lipase-alpha [DAGL-alpha]); major endocannabinoids (anandamide [AEA], 2-arachidonylglycerol [2-AG]); lipid precursors and metabolites (arachidonic acid [AA], 2-acylglycerol [AG], diacylglycerol [DAG], and ethanolamine [$EtNH_2$]); cannabinoid receptor 1 (CB_1); neurotransmitter (NT); and major signaling cascade mediators downstream of CB_1 activity (mitogen-activated protein kinases [MAPK], adenylate cyclase [AC], and calcium [Ca^{2+}] signaling). Endocannabinoids signal in a retrograde manner to activate presynaptic CB_1, which mediates signaling mechanisms that influence synaptic transmission and neurotransmitter release.

The Endocannabinoid Pathway and Alcohol Interactions

There is a high degree of comorbidity between AUD and CUD, which indicates a functional link between alcohol and cannabis.[18] Synergistic effects also have been observed in rodents. For instance, co-administration of ethanol and cannabinoids has additive effects on some behaviors such as sleep,[69] cognitive, psychomotor, and attention deficits.[70] Additionally, alcohol and cannabis use might cause cross-tolerance,[18,71] and acute tolerance of alcohol is thought to be mediated through the eCB system.[72] Synergistic behaviors are reviewed by Pava and Woodward,[18] Basavarajappa et al.,[19] Kunos,[20] and Henderson-Redmond et al.[73]

Although the focus of this review is the synaptic mechanisms of eCBs and alcohol, a brief description of the behavioral implications is provided for context throughout. The eCB system has emerged as a promising druggable target for the development of therapeutic options to treat AUD. Pharmacological modulation of the eCB system by CB receptor agonists, antagonists, eCB-degrading enzyme inhibitors, or anandamide transporter inhibitors alters the alcohol-related behaviors in rodents. Rats treated with CB_1 antagonist SR 141716A (rimonabant), or its analog surinabant (SR 147778), showed reduced alcohol consumption and motivation to consume alcohol in various drinking models.[74-79] CB_1 agonists WIN 55,212-2 (WIN) and CP 55,940 increased ethanol consumption and preference in mice and rats.[80,81] Activation of CB_2 signaling using the agonist JWH133 seems to reduce both alcohol- and food-rewarding behaviors.[82] The expression and function of CB_1 receptors and FAAH are altered in AUD,[83,84] and pretreatment with the FAAH inhibitor URB597 reduced alcohol intake and preference after acute withdrawal through a CB_1-mediated mechanism.[85] However, URB597 administration increased operant ethanol self-administration in rats,[84] whereas AEA transport blocker AM404 had efficacy in reducing ethanol self-administration in rodent models.[86] The discrepancy between the effects of the FAAH inhibitor URB597[84] and the AEA transport blocker in models of alcohol self-administration might be due to the mechanism of action of AM404,[86] which does not involve the CB_1 receptor, given that the administration of CB_1 antagonists or agonists does not affect alcohol self-administration.[86] Interestingly, recent findings from Soria-Gomez et al. have shown that the activation of CB_1 at different subcellular locations (plasma membrane vs. mitochondria) within the same circuit is associated with opposite behavioral outcomes.[87] This observation might shed light on why alcohol often has discrepant effects on the activation or inhibition of the eCB system and vice versa.[87]

Ethanol and cannabinoids induce neurophysiological consequences through their interaction with specific substrates (i.e., receptors and enzymes). Although cannabinoids primarily modulate synaptic neurotransmission via the eCB system, ethanol interacts with a variety of different molecular substrates that affect a diverse range of neurochemical processes. The eCB system plays a critical role in mediating the effects of ethanol in the brain, contributing to ethanol-induced biochemical, genetic, electrophysiological, and behavioral consequences. This suggests that eCB signaling contributes to the underlying neuropathology that drives AUD.[18] Despite this strong brain implication, the synaptic mechanisms of alcohol and eCB signaling are still not fully investigated, and some brain regions involved in the addiction cycle are relatively unexplored. Additionally, alcohol paradigms vary across studies, and acute, chronic, and withdrawal exposures are not fully characterized within specific brain regions. Therefore, the following discussion of the current literature on synaptic eCB and alcohol interactions is divided into two main sections: (1) acute alcohol exposure and (2) chronic alcohol exposure and withdrawal. Each section is subdivided by brain region—where data are available—including the hippocampus, amygdala, prefrontal cortex, basolateral amygdala (BLA), nucleus accumbens (NAc), ventral tegmental area (VTA), striatum, and cerebellum.

Acute Alcohol Exposure and eCB System Interactions

Acute alcohol exposure produces intoxicating effects by acting on the CNS, both at low and high concentrations (1–100 mM) in preclinical animal or cell culture experiments and nontolerant humans.[88] Acute concentrations of ethanol can directly interact with several molecules and have specific effects on different brain regions.[89] Ethanol has rapid acute effects on the function of proteins involved in excitatory and inhibitory synaptic transmission.[88] Some of these effects are mediated by eCB signaling and subsequent alterations in neurotransmission and synaptic activity. However, the eCB system is complex, and ethanol-induced effects are brain region–specific and sensitive to the exposure methodology used. Therefore, discrepancies between studies occur, possibly because of differences in methodology, tissue/cell culture, and ethanol exposure paradigm.

Hippocampus

Acute alcohol exposure is known to affect hippocampal function and to impact contextual and episodic memory by altering neuronal processes.[90] In general, acute alcohol exposure consistently decreases eCB (AEA, 2-AG) levels as measured directly in tissue of the striatum, hippocampus, prefrontal cortex, amygdala, and cerebellum.[91-93] The decreases in eCBs observed are not due to increased metabolism by FAAH activity and therefore are not mediated by metabolic activity and degradation of eCBs.[91] Furthermore, FAAH activity in the hippocampus was transiently decreased 45 minutes post intraperitoneal (IP) injection of ethanol (4 g/kg).[91] However, as

stated earlier, discrepancies between studies occur, possibly due to methodology, differences in tissues/cell cultures, and ethanol exposure paradigm. For example, in contrast to the above studies, acute alcohol exposure in hippocampal neurons increased both AEA and 2-AG levels via a calcium-dependent mechanism and subsequently inhibited presynaptic glutamate release.[94] Acute ethanol exposure did not alter CB_1 presynaptic expression but did enhance both AEA and 2-AG.[94] Ethanol-induced alterations in CB receptor activity and eCB levels affect the eCB system and may lead to disruptions in synaptic function. Ethanol decreases the frequencies, but not amplitude, of spontaneous miniature excitatory postsynaptic currents (mEPSCs), suggesting inhibition of vesicular glutamate release and suppression of synaptic functions.[94] These studies overall demonstrate the complex role of eCB signaling in regulating ethanol-induced effects in the hippocampus.

Cannabinoids and acute alcohol exposure alter synaptic transmission in the hippocampus through the eCB system. Specifically, cannabinoid exposure inhibited glutamatergic synaptic transmission in hippocampal cultures[95] and inhibited calcium currents in cell cultures.[96] In rat hippocampal cultures, the cannabimimetic WIN inhibited N- and P/Q-type calcium channels through the CB_1 receptor whereas the nonpsychoactive enantiomer, WIN 55,212-3, was not effective. Maximal inhibition by the nonclassical cannabinoid agonist CP 55,940 was similar to that seen with maximal concentrations of WIN.[97]

Amygdala

The extended amygdala represents a macrostructure composed of several basal forebrain structures: the bed nucleus of the stria terminalis, central medial amygdala (CeA), and a transition zone in the posterior part of the medial NAc (i.e., posterior shell).[98-100] Key elements of the extended amygdala include not only neurotransmitters associated with the positive reinforcing effects of substances such as alcohol, opioids, cocaine, and amphetamines, but also major components of the brain stress systems associated with the negative reinforcement of drug dependence.[100-102] CB_1 in part regulates the effects of alcohol in CeA neurons, and activation of CB_1 attenuates the alcohol effect on the CeA's gamma-aminobutyric acid (GABA) system.[11] Acute application of ethanol in an ex vivo CeA brain slice induced presynaptic facilitation of GABAergic signaling on rat CeA neurons via increased GABA release.[103-105] This ethanol-induced, evoked, and spontaneous GABA release was blocked by CB_1 activation via the agonist WIN.[54,55] Similarly, superfusion of WIN prevented subsequent ethanol effects on GABAergic transmission. The application of CB_1 antagonists rimonabant and AM251 alone augmented GABAergic responses, revealing a tonic eCB activity that decreased inhibitory transmission in CeA via a presynaptic CB_1 mechanism. The intracellular calcium chelator BAPTA abolished the ability of AM251 to augment GABA responses, demonstrating the eCB-driven nature and postsynaptic origin of the tonic CB_1-dependent control of GABA release. Notably, the ethanol-induced facilitation of GABA release was additive to CB_1 blockade, ruling out participation of CB_1 in the action of acute ethanol.[54,55] These studies on both evoked and spontaneous GABA transmission point to an important role of CB_1 in the CeA, in which the eCBs tonically regulate neuronal activity and suggest a potent mechanism for modulating CeA tone during challenge with ethanol.[54]

CB_1 activation is known to decrease glutamate release in many brain areas, including the CeA, of male rodents.[51,106] Glutamatergic transmission also was investigated in the CeA of Wistar and Marchigian Sardinian alcohol-preferring (msP) rats.[107] Notably, msP rats display enhanced anxiety, stress, and alcohol drinking, simulating the alcohol-dependent phenotype. Findings indicate that acute ethanol application decreases evoked excitatory postsynaptic potential amplitudes in rat CeA. WIN decreased glutamatergic responses via presynaptic mechanisms in male rats only, and combined application of WIN and acute ethanol exposure resulted in strain-specific effects in females.[107] No tonic CB_1 signaling at glutamatergic synapses in the CeA of any groups, and no interactions with ethanol were observed. Collectively, these observations demonstrate sex strain–specific differences in ethanol and endocannabinoid effects on CeA glutamatergic signaling.[107]

Basolateral amygdala

The eCB system in the BLA plays a role in gating stress and anxiety responses by modulating GABA and glutamate transmission.[108,109] CB_1 is highly expressed in cholecystokinin-positive GABAergic interneurons[110,111] and at lower levels in glutamatergic pyramidal cells.[111] A wide body of work has demonstrated that CB_1 activity decreases GABAergic transmission in the BLA.[110,112-114] GABAergic transmission in the BLA is increased by acute ethanol exposure in naïve rats via both presynaptic and postsynaptic mechanisms. Although CB_1 activation impairs ethanol's facilitation of GABAergic transmission, ethanol's presynaptic site of action is likely independent of CB_1, given that acute ethanol application further increases GABA release in the presence of a CB_1 antagonist.[115] CB_1 antagonism with rimonabant or chronic pretreatment with CB_1 agonist WIN attenuates acute alcohol-induced inhibition of neuronal firing in the BLA.[116] Further evidence shows that eCBs are either not released or cannot activate CB_1 receptors in the presence of ethanol, resulting in GABAergic transmission under conditions when they would normally be suppressed.[117] Interestingly, ethanol prevented depolarization-induced suppression of inhibition even when the postsynaptic neuron was loaded with AEA during the experiment, suggesting that increasing the eCBs available for release could not overcome the ethanol effect.[117]

Nucleus accumbens

The NAc mediates emotional and reward-related stimuli by integrating signals from the limbic system.[101,118,119] In the NAc, acute ethanol altered eCB system components, which may affect NAc function. Acute alcohol IP administration (15% ethanol, 4 g/kg) increased AEA and CB_1 binding in rat NAc[120] and in immature mouse hippocampus and cortex.[121] Therefore, acute alcohol enables eCB synthesis and release.[94,116] Self-administration of ethanol (10% for 30 minutes) by rats acutely increased 2-AG interstitial levels in the NAc shell during ethanol exposure with no concurrent alteration in AEA, as measured by in vivo microdialysis. Interestingly, the relative change in dialysate 2-AG levels was positively correlated with the amount of ethanol consumed.[122]

In the NAc, acute ethanol exposure enhances dopamine release, which can be inhibited by blockade or genetic ablation of CB_1, suggesting that acute alcohol exposure facilitates the dopaminergic system via the eCB system.[123] In awake, freely moving rats, acute ethanol treatment (IP injection) induced a dose-dependent release of dopamine in the dopaminergic projection area of the NAc.[124] This ethanol-induced release of dopamine was exacerbated in alcohol-preferring rats when compared to alcohol-avoiding rats.[125] With CB_1 activation (via THC or WIN), dopamine release was elicited in the rat NAc shell similarly to that induced by alcohol,[126] and CB_1 activity induced an increase in spontaneous firing due to inhibition of GABAergic inputs onto projections of dopaminergic neurons to the NAc (see the VTA section below for detail).[127-129] Modulation of the dopamine system in the NAc is complex, and activation of CB_1 on prefrontal cortex glutamatergic terminals in the NAc reduces glutamatergic transmission and consequently dopamine. This may limit the rewarding effects of acute alcohol exposure.[130]

Ventral tegmental area

The VTA is known to mediate the positive reinforcement effect of alcohol. Dopaminergic neurotransmission in the VTA was identified as a key mechanism for the establishment and maintenance of alcohol intake.[131] Similar to the NAc, acute alcohol exposure increased the firing rate of VTA dopaminergic neurons in a CB_1-dependent manner.[17] CB_1 is not expressed on dopaminergic neurons in the VTA; therefore, the eCB-induced increase in dopamine release in the VTA is mediated by CB_1 activity on inhibitory GABAergic interneurons. This results in disinhibition of dopaminergic neurons in the VTA and increased dopamine release in the NAc.[128,129]

Striatum

The striatum is implicated in habit formation and motivation or goal-directed actions, and acute alcohol exposure disrupts the stability of striatal neuronal circuits.[132] In the striatum, the physiological effects of acute ethanol exposure appear to oppose, or are antagonized by, eCB signaling mechanisms. In the rat dorsomedial striatum, acute alcohol exposure inhibited eCB release from medium spiny neurons, preventing lasting disinhibition. This effect was found to be independent of eCB synthesis and CB_1 activity. In the rat dorsomedial striatum, release of eCBs from medium spiny neurons is associated with disinhibition of these neurons for an extended period of time and decreased synaptic long-term depression. This long-lasting disinhibition can be blocked independently of CB_1 activation or synthesis of eCBs by pretreatment with alcohol. Acute ethanol treatment prevents the long-lasting disinhibition induced by the CB_1 agonist WIN in rat striatum. These data suggest that the eCB system is involved in the physiological response to acute alcohol intoxication.[132]

Cerebellum

Cerebellum function can be affected by alcohol, causing disruptions in locomotion, balance, and executive functions. Acute alcohol exposure impairs cerebellar function by altering gamma-aminobutyric acid type A ($GABA_A$) receptor-mediated neurotransmission.[133] Ethanol induces presynaptic GABA release onto cerebellar Purkinje neurons through a pathway that is dependent on protein kinase A (PKA) and that releases calcium from internal stores independent of eCB synthesis.[134] In contrast, activation of CB_1 in Purkinje neurons inhibits the ethanol-induced GABA release from presynaptic terminals and the frequency of inhibitory postsynaptic currents (IPSCs). This blockade of ethanol-induced IPSC frequency is mediated by the PKA pathway, through G protein (G_i)-mediated inhibition of PKA produced by activation of CB_1.[135] Notably, CB_1 activation by WIN also blocked ethanol from increasing spontaneous GABA release onto the interneuron–Purkinje cell synapses in the cerebellum.[135]

Summary

The above studies (summarized in Table 1) indicate that acute alcohol exposure profoundly affects the eCB system, including expression and function of eCB signaling components that subsequently impact neuronal function and synaptic transmission. It is also evident that acute ethanol exposure differentially affects the eCB system depending on brain region and alcohol administration method. Further difficulties in elucidating alcohol and the eCB system interactions arise from the complexity of the eCB pathway due to its retrograde signaling on both GABAergic and glutamatergic synapses.[20,29,32,41] Additionally, factors such as the state of tissue or cells under study (ex vivo, in vivo, or in vitro) or the species (mice or rats) may affect results.[18] Although alcohol-related behavioral studies implicate the importance of the eCB system, the underlying effects induced by acute ethanol exposure on the synaptic interactions between alcohol and the endogenous cannabinoid system are not well understood.

Table 1. Acute Ethanol Exposure and ECB System Interaction, by Brain Region and Study

Brain Region and Study	Ethanol Exposure	System	Species	Measure	Effect	Drug	Synaptic Activity	Effect
Hippocampus								
Ferrer et al. (2007)[91]	4 g/kg, IP	Tissue	Wistar rats	AEA, 2-AG	Decrease			
Rubio et al. (2009);[92] Rubio et al. (2007)[93]	24h liquid diet	Tissue	Sprague-Dawley rats	AEA, 2-AG	Decrease			
Ferrer et al. (2007)[91]	4 g/kg, IP	Tissue	Wistar rats	FAAH activity	Decrease			
Basavarajappa et al. (2008)[94]	30 and/or 60 min, 50 mM	Cultured neurons	C57BL/6J mice	AEA, 2-AG CB_1 expression Presynaptic glutamate release	Increase No change Inhibition			
Amygdala								
Roberto et al. (2004);[103] Roberto et al. (2004);[104] Roberto et al. (2003)[105]	5–10 min, 44 mM	Brain slice	Sprague-Dawley rats	GABA transmission	Increase			
Roberto et al. (2010);[54] Varodayan et al. (2016)[55]	5–10 min, 44 mM	Brain slice	Sprague-Dawley rats			WIN	Evoked and spontaneous GABA responses	Blockade
Roberto et al. (2010);[54] Varodayan et al. (2016)[55]	5–10 min, 44 mM	Brain slice	Sprague-Dawley rats			Rimonabant, AM251	Evoked and spontaneous GABAergic responses	Increase
Kirson et al. (2018)[107]	10–15 min, 44 mM	Brain slice	Wistar and msP rats	Glutamatergic transmission	Decrease	WIN AM251	Evoked glutamatergic response (evoked EPSCs)	Further inhibition (males) and blockade of ethanol effect (Wistar females) with WIN No change with AM251
Basolateral amygdala								
Varodayan et al. (2017)[115]	5–10 min, 44 mM	Brain slice	Sprague-Dawley rats	GABAergic transmission	Increase	WIN AM251	Spontaneous GABAergic transmission (GABA release)	Reduction with WIN Increase with AM251
Perra et al. (2008)[116]	0.25–2.0g/kg, IV	Brain slice	Sprague-Dawley rats			Rimonabant, WIN chronic pretreatment	Inhibition of neuronal firing by ethanol	Reduction

Table 1. Acute Ethanol Exposure and ECB System Interaction, by Brain Region and Study (Continued)

Brain Region and Study	Ethanol Exposure	System	Species	Measure	Effect	Drug	Synaptic Activity	Effect
Nucleus accumbens								
Ceccarini et al. (2013)[120]	4 g/kg, IP	Tissue	Wistar rats	AEA, CB_1 binding	Increase			
Caillé et al. (2007)[122]	30 min self-administration	Dialysate	Wistar rats	2-AG AEA	Increase No change			
Hungund et al. (2003)[123]	1.5 g/kg, IP, 20–280 min	Dialysate	Mice	Dopamine release	Increase	CB_1 knockout, Rimonabant	Dopamine release with ethanol	Inhibition
Di Chiara et al. (1988)[124]	0.25–2.5 g/kg, IP	Dialysate	Sprague-Dawley rats	Dopamine release	Increase			
Ventral tegmental area								
Perra et al. (2005)[17]	0.5 g/kg, IV	Brain slice	Sprague-Dawley rats	Dopaminergic neurons firing	Increase			
Striatum								
Clarke et al. (2009)[132]	20 min, 20–50 mM	Brain slice	Wistar rats	eCB release	Inhibition and prevention of long-lasting neuronal disinhibition			
Cerebellum								
Kelm et al. (2007)[134]	5 min, 50–100 mM	Brain slice	Sprague-Dawley rats	Presynaptic GABA release	Increase			
Kelm et al. (2008)[135]	5 min, 50–100 mM	Brain slice	Sprague-Dawley rats			WIN	Presynaptic GABA release (sIPSCs)	Inhibition

Note: 2-AG, 2-arachidonoylglycerol; AEA, arachidonoylethanolamide (anandamide); CB_1, cannabinoid receptor 1; eCB, endocannabinoid; EPSCs, excitatory postsynaptic currents; FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; IP, intraperitoneal; IV, intravenous; sIPSCs, spontaneous inhibitory postsynaptic currents; WIN, WIN 55,212-2.

The eCB System in Chronic Alcohol Exposure and Alcohol Withdrawal

Chronic ethanol exposure induces many neuroadaptive changes in the CNS involving both GABAergic and glutamatergic synaptic transmission. Long-term ethanol exposure results in both tolerance and dependence. Tolerance presents as a decreased behavioral response to ethanol and decreased intoxication. Dependence is described by symptomatology elicited during and following ethanol withdrawal, including anxiety, hyperalgesia, dysphoria, susceptibility to seizures, and disrupted sleep states.[88] Both chronic ethanol and cannabinoid exposure produce similar adaptations in eCB signaling.[10] Cross-tolerance with alcohol and cannabis also is consistent with changes in CB_1 expression.[18] Preclinical studies using different chronic ethanol treatment models have consistently observed reduced CB_1 expression or function in a variety of rodent brain regions[136-139] and in alcohol-preferring rats.[140-142] However, as with acute exposure to alcohol, effects of chronic alcohol exposure may vary depending on exposure paradigm and may be brain region–specific. In humans, chronic heavy drinking (defined as greater than six drinks per day, where a standard drink contains ~ 10g of ethanol) is linked to reduced CB_1 receptor availability and binding in numerous brain regions that persist after prolonged abstinence or withdrawal, and amount of alcohol intake is negatively correlated with years of misuse.[137,143] Chronic dysregulation of the eCB system suggests a mechanism underlying the negative affect associated with AUD.[20] Although the effects of alcohol withdrawal on the eCB pathway are not well known, alcohol withdrawal in some cases recovers the effects induced by chronic alcohol exposure on components of the eCB system.[120,136,144-147]

Hippocampus

Chronic ethanol exposure induced structural and functional changes in the hippocampus.[118,148,149] This region is also highly sensitive to the damaging effects of chronic alcohol use.[90] Multiple studies demonstrate that chronic alcohol exposure and withdrawal dysregulate the hippocampal eCB system. Regional dysfunction was identified in CB_1, indicated by reduced relative CB_1 binding, in the hippocampus and caudate-putamen of rats exposed to alcohol via liquid diet for 7 days.[120] A 7-day alcohol paradigm reduced WIN sensitivity and induced altered monoamine synthesis in the locus coeruleus, hippocampus, and striatum.[150] Additionally, genetic deletion of CB_1 impaired the neuroadaptations of NMDA and $GABA_A$ receptors in the cerebral cortex and hippocampus induced by chronic ethanol treatment, indicating that the eCB system plays a critical role in alcohol dependence.[151]

Alcohol-dependent rats (52 days of forced access) were found to have reduced CB_1 gene expression (measured via *Cnr1* messenger RNA [mRNA] levels) in the hippocampus, hypothalamus, and striatum.[141] Similarly, chronic intermittent ethanol (CIE) exposure via oral intubation (55 days of forced access followed by 2 days of withdrawal) in rats reduced *Cnr1* expression and CB_1 levels in the hippocampus.[139] In alcohol-preferring msP rats, *Cnr1* expression was greater in several brain regions including the frontoparietal cortex, caudate-putamen, and hippocampus, although this was reversed following alcohol self-administration.[140] Sardinian alcohol-preferring (sP) rats, compared to alcohol–non-preferring rats, display greater CB_1 density, *Cnr1* levels, and eCB levels in the cerebral cortex, hippocampus, and striatum. Reduced FAAH expression also was observed in the hippocampus of sP rats.[147] Consistent with these findings, 12 weeks of CIE vapor reduced *Cnr1* and CB_1 levels in the rat lateral habenula, while enhancing levels of the eCB-related mRNA and/or proteins, DAGL-beta, NAPE-PLD mRNA (*napepld*), and MAGL.[152] In contrast, no change in CB_1 receptor binding and mRNA levels occurred in the hippocampus, cerebral cortex, or motor and limbic structures in a chronic ethanol intake model (7% liquid diet for 15 days).[153]

The eCB system's role in alcohol withdrawal in the hippocampus is not well understood, and studies are variable. The dysfunction in CB_1 identified by Ceccarini et al. was reversed after 2 weeks of abstinence from alcohol.[120] However, another study identified lasting effects on eCBs; even with 40 days of withdrawal, alcohol-dependent rats retained enhanced AEA and 2-AG levels in the hippocampus.[139] Despite this molecular evidence, synaptic studies on the functional consequences of the changes observed in eCBs are lacking.

Prefrontal cortex

Chronic alcohol exposure affects the structure and function of the prefrontal cortex, causing deficits in executive control, decision-making, and risk management.[154] As observed in the hippocampus, chronic alcohol exposure induces alterations in NMDA and $GABA_A$ receptor expression in wildtype mice, but not in CB_1-depleted mice, indicating that the eCB system plays a critical role in alcohol dependence.[151] Additionally, in situ hybridization in msP rats identified that *Cnr1* expression is greater in the frontoparietal cortex; this was reversed following alcohol self-administration.[140] However, no change in CB_1 receptor binding and mRNA levels occurred in the cerebral cortex with chronic ethanol intake (7% liquid diet for 15 days).[155]

Acute application of the CB_1 agonist WIN enhanced the amplitude of the period of depolarization (up states) in slice cultures of the prefrontal cortex but not in slices that underwent 10 days of chronic ethanol treatment followed by 4 days of withdrawal. Chronic ethanol followed by 4 days of withdrawal blunted WIN inhibition of evoked GABA inhibitory postsynaptic currents (IPSCs) in layer II/III of the pyramidal neurons but not in layer V/VI. WIN inhibited the amplitude of spontaneous GABA IPSCs in both layers and this effect was not altered by ethanol

treatment.[144] Some studies indicate that alcohol withdrawal may lessen the effects of eCB system alterations induced by chronic alcohol exposure. CIE exposure increased *Cnr1* expression in the medial prefrontal cingulate cortex, and alcohol withdrawal recovers the effects of chronic exposure to control levels in rats.[145] Acute alcohol withdrawal also produced reduction in gene expression of components of the eCB system and reduced 2-AG content in the medial prefrontal cortex of male rats, but not in female rats.[146]

Amygdala

In the amygdala, eCB signaling is compromised in alcohol-dependent animal models. Chronic alcohol intake in rats (7% liquid diet for 15 days) induced a decrease in both 2-AG and AEA in the midbrain and an increase in AEA in the limbic forebrain, but no change occurred in CB_1 receptor binding and mRNA levels in limbic structures.[136,153,155] A chronic ethanol liquid diet (10% ethanol, continuous access for 15 days; or intermittent access for 5 days/week for 3 weeks) followed by acute withdrawal (6 or 24 hours) significantly altered gene expression for a variety of components of the amygdala's eCB system. Reductions in FAAH, MAGL, CB_1, CB_2, and GPR55 mRNA were observed, with alteration in MAGL and CB receptor–associated mRNA being more pronounced with intermittent alcohol exposure.[156] In the CeA, an alcohol self-administration paradigm decreased 2-AG levels in dependent rats, and MAGL inhibitors increased alcohol consumption.[157] In baseline CeA dialysate, AEA and 2-AG levels decreased in ethanol-dependent rats with further decrements during 12-hour withdrawal. Subsequent ethanol consumption restored 2-AG dialysate content to baseline levels.[157,158] MsP rats also displayed higher FAAH activity and decreased AEA levels in the CeA as measured by microdialysis.[142]

GABAergic dysregulation in the CeA is a hallmark of the transition to alcohol dependence in animal models.[101] A study by Varodayan and colleagues reported that activation of CB_1 via WIN decreased the frequency of spontaneous and miniature CeA $GABA_A$ receptor-mediated IPSCs, which could be blocked by CB_1 antagonism.[55] Two weeks of CIE vapor significantly blunted this effect of WIN. Chronic ethanol exposure abolished tonic CB_1 influence on vesicular GABA release, indicating that CB_1 function in the CeA is impaired by chronic ethanol exposure.[55] Therefore, decreased CB_1 activity is likely a factor that contributes to the dysregulated (enhanced) GABA transmission in the CeA with chronic alcohol exposure.[55] Altered eCB function may contribute to the dependence-associated disruptions in glutamate and GABA transmission in the CeA.[11,103] These findings indicate that eCB signaling is compromised in the amygdala of ethanol-dependent rats, contributing to an allostatic shift toward maintenance of ethanol intake through negative reinforcement.[34,54,158]

Basolateral amygdala

Chronic ethanol exposure and withdrawal alter synaptic transmission in the BLA.[114,116,159-161] Emotional processing is affected by the actions of CB_1 on GABA and glutamate neurotransmission in the BLA.[108-110,112-114,162,163] Decreased CB_1 and increased AEA levels were observed in the BLA with a 10-day CIE vapor paradigm.[164] Additionally, ethanol exposure caused a dose-dependent inhibition of glutamatergic synaptic activity via a presynaptic mechanism that was occluded by CB_1 antagonists rimonabant and AM251. Importantly, this acute ethanol inhibition was attenuated following CIE.[164] Withdrawal produced a reduction in the gene expression of *Cnr1* and the protein levels of DAGL-alpha, MAGL, and AEA levels in the BLA of male rats, but not female rats.[146] In naïve rats, WIN application decreased GABA release, which was prevented by CB_1 antagonist AM251. AM251 increased GABA release via a postsynaptic, calcium-dependent mechanism. This retrograde tonic CB_1 signaling was reduced in rats exposed to 2 weeks of CIE, suggesting impaired eCB signaling. These results indicate that CB_1 has a critical role in regulating BLA GABAergic transmission, which is dysregulated with chronic ethanol exposure.[115]

Ventral tegmental area

Few studies have investigated chronic alcohol exposure in the VTA. However, one study conducted in mice identified that VTA $GABA_A$ receptor inhibition in dopaminergic neurons was regulated through presynaptic actions of eCBs. The same study showed that withdrawal from CIE vapor exposure increased eCB-mediated inhibition on GABA synapses of VTA dopamine neurons.[165] Withdrawal was shown to decrease sensitivity to WIN and enhance sensitivity to AM251, suggesting that $GABA_A$ inhibition of dopamine neurons in the VTA is regulated by presynaptic eCB activity and that withdrawal increases eCB-mediated inhibition.[165]

Striatum

In the rat striatum, chronic alcohol treatment is associated with dysregulation of the eCB system, specifically with a decrease in *Cnr1* mRNA levels.[140,141] Similar to the cortex, hippocampus, and cerebellum, a 72-hour ethanol vapor inhalation paradigm decreased CB_1 receptor density and CB_1 activation in mouse striatum. These effects were recovered after 24 hours of withdrawal from ethanol, suggesting that these eCB neuroadaptations may play a role in development of tolerance and dependence.[136,147] In sP rats, greater CB_1 density, CB_1 mRNA, CB_1-mediated G protein coupling, and eCB levels were

observed in the striatum. Alcohol intake (homecage two-bottle free-choice regimen with unlimited access for 24 hours/day for 70 consecutive days) in sP rats reduced CB_1-mediated G protein coupling, which was reversed by rimonabant administration, and increased eCBs in the striatum, associating the eCB system with higher alcohol preferences.[147] Studies in humans also identified altered eCB signaling components. Human postmortem tissue from patients with AUD has decreased CB_1 expression, decreased FAAH expression and activity, and increased AEA levels, all specifically identified in the ventral striatum.[166]

Additionally, synaptic plasticity may be influenced by ethanol and mediated via the eCB system. CIE vapor in mice abolished CB_1-mediated long-term depression in the mouse dorsolateral striatum and increased 2-AG.[167] These results suggest that chronic ethanol exposure causes neuroadaptations in the striatum that may contribute to the progression of AUD in humans and alcohol dependence in animals.[167]

Cerebellum

Analogous to acute exposure, chronic alcohol exposure disrupts cerebellar function through $GABA_A$ and eCB mechanisms.[133] As in the striatum, chronic ethanol exposure decreased CB_1 receptor density and activity in the mouse cerebellum, which was reversed with withdrawal.[136] In cultured cerebellar granular neurons and cultured neuronal cells (human neuroblastoma SK-N-SH), 72 hours of ethanol exposure increased the synthesis of AEA and 2-AG through calcium activation of phospholipase A2 and subsequently increased NAPE-PLD activity in cultured cells.[19,138,168] Additionally, in mouse synaptic plasma membrane, chronic alcohol exposure decreased the function and expression of CB_1.[138,169,170] Similarly, chronic alcohol intake induced an increase in AEA levels and a decrease in components of AEA transport and FAAH in cultured cerebellar neurons.[171]

Summary

Overall, these data (summarized in Table 2) indicate that chronic alcohol exposure compromises CB_1 and eCB pathways, and alcohol withdrawal may ameliorate these effects. The chronic alcohol-induced molecular changes in the eCB system—including the synthesis of eCBs and the expression of CB_1 and catabolizing enzymes—have a profound impact on neuronal function and synaptic transmission in multiple brain regions.[13,155] These effects with alcohol withdrawal may be due to a compensatory effect to regulate neurotransmission and counteract neuroadaptations induced with chronic alcohol exposure. The strong association of polydrug use with alcohol and cannabis products presents the possibility of self-medicating for AUD with cannabis and developing CUD.[18,172,173] Further research on the eCB pathways may facilitate the modulation of the eCB system as a target for future AUD treatment.

General Summary and Future Directions

There is clear evidence that the eCB system plays a critical role in the acute effects of alcohol on synaptic functions, and that neuroadaptations occur with chronic alcohol exposure and withdrawal in eCB signaling. The eCB system orchestrates a complex signaling mechanism. Ethanol- and/or withdrawal-induced molecular alterations in the eCB system impact neuronal functions and synaptic transmission in a brain region–specific manner. A variety of studies have demonstrated the potential beneficial effects of several pharmacological approaches for treating AUD by modulating the eCB system.[84,156,157,174] A growing number of CB_1 and CB_2 agonists and antagonists, FAAH and MAGL inhibitors, as well as NAPE-PLD and DAGL inhibitors have been developed in the past 2 decades. However, determining how ethanol exposure affects eCB metabolizing enzymes at the synaptic level requires further research and will provide invaluable insight to guide our understanding of the pathophysiology of alcohol-induced synaptic changes. Specifically, FAAH and MAGL inhibitors have been proven efficacious in ameliorating the negative affect in preclinical models of AUD.[157,174-177] However, more research is needed to understand how these compounds affect synaptic transmission.

Many studies have identified the importance of eCB signaling in mediating behavioral responses to alcohol exposure and withdrawal; however, the underlying neuronal mechanism is not well characterized. Unfortunately, the current literature is limited and lacks the consistency (length of ethanol exposure, time of measurements, neurochemicals measured, etc.) across brain regions that is necessary for a more comprehensive understanding of the synaptic interactions of the eCB system and alcohol. However, a few studies that are consistent indicate strong themes within brain regions. For instance, a variety of chronic ethanol exposure paradigms in the hippocampus consistently indicated a reduction in CB_1 function, assessed via CB_1 gene expression,[139,141] binding,[120] and WIN sensitivity,[150] in most studies and in multiple rat strains.[140,147] In studies where a similar methodology is used, such as in the amygdala, strong and consistent evidence identified the role of CB_1 in the effects of acute alcohol exposure.[11,54,55] CB1 was found to attenuate the acute ethanol-induced facilitation of GABAergic signaling in the CeA.[54,55] Combined, these studies identified an important role of the eCB system in modulating CeA signaling during alcohol exposure. However, in many cases, studies and research are insufficient to draw a detailed and comprehensive consensus of the synaptic role of the eCB system within different alcohol stages and brain regions. From the review of

Table 2. Chronic Ethanol Exposure, Withdrawal, and ECB System Interaction, by Brain Region

Brain Region and Study	Ethanol Exposure	System	Species	Measure	Effect	Drug	Synaptic Activity	Effect
Hippocampus								
Ceccarini et al. (2013)[120]	7 days liquid diet (7% v/v)	Tissue	Wistar rats	CB_1 binding	Reduction			
Ceccarini et al. (2013)[120]	7 days liquid diet (7% v/v) + 2 weeks abstinence	Tissue	Wistar rats	CB_1 binding	Recovery			
Ortiz et al. (2004)[141]	52 days forced access	Tissue	Wistar rats	CB_1 gene expression	Reduction			
Mitrirattanakul et al. (2007)[139]	55 days oral intubation (6 g/kg daily) + 2 days withdrawal	Tissue	Sprague-Dawley rats	CB_1 gene expression, CB_1 protein	Reduction			
Cippitelli et al. (2005)[140]	30 min daily sessions on a fixed ratio 1 schedule of reinforcement self-administration	Tissue	msP rats	CB_1 gene expression	Reduction			
González et al. (2002)[153]	15 days liquid diet (7% v/v)	Tissue	Wistar rats	CB_1 binding and gene expression	No change			
Mitrirattanakul et al. (2007)[139]	55 days oral intubation (6 g/kg daily) + 40 days withdrawal	Tissue	Sprague-Dawley rats	CB_1 gene expression, CB_1 protein, AEA, 2-AG	Increase			
Prefrontal cortex								
Cippitelli et al. (2005)[140]	18 days self-administration (10% v/v in 30 min daily sessions on a fixed ratio 1 schedule reinforcement)	Brain slice	msP rats (and Wistar rats)	CB_1 gene expression	Reduction			
González et al. (2002)[155]	15 days liquid diet (7% v/v)	Tissue	Wistar rats	CB_1 binding and gene expression	No change			
Pava et al. (2014)[144]	4 days withdrawal after 10 days chronic ethanol	Slice cultures	C57BL6/J mice			WIN	Spontaneous GABA transmission	No change
Rimondini et al. (2002)[145]	7 weeks intermittent alcohol (17 h/day)	Tissue	Wistar rats	CB_1 gene expression	Increase			
Rimondini et al. (2002)[145]	3 weeks after 7 weeks of intermittent alcohol	Tissue	Wistar rats	CB_1 gene expression	Recovery			
Henricks et al. (2017)[146]	Acute (1-4 days) withdrawal after 6 weeks chronic intermittent alcohol vapor	Tissue	Wistar rats	2-AG	Reduction			

Table 2. Chronic Ethanol Exposure, Withdrawal, and ECB System Interaction, by Brain Region (Continued)

Brain Region and Study	Ethanol Exposure	System	Species	Measure	Effect	Drug	Synaptic Activity	Effect
Amygdala								
González et al. (2002)[153,155]	15 days liquid diet (7% v/v)	Tissue	Wistar rats	AEA CB_1 binding and gene expression	Increase No change			
Serrano et al. (2012)[156]	Withdrawal after 5 days per week for 3 weeks	Tissue	Wistar rats	CB_1, MAGL gene expression	Reduction			
Serrano et al. (2018)[157]	30 min on a fixed ratio 1 schedule self-administration	Dialysate	Wistar dependent rats	2-AG	Decrease			
Serrano et al. (2018);[157] Chevaleyre et al. (2006)[158]	12 h withdrawal	Dialysate	Wistar dependent rats	AEA, 2-AG	Decrease			
Varodayan et al. (2016)[55]	2–3 weeks CIE vapor for 14 h a day	Brain slice	Sprague-Dawley rats			WIN, AM251	Spontaneous GABA transmission (GABA release)	CIE blunts WIN effect
Basolateral amygdala								
Robinson et al. (2016)[164]	10 days CIE vapor	Tissue; Brain slice	Sprague-Dawley rats	AEA CB_1	Increase Decrease		Glutamatergic transmission	Inhibition
Robinson et al. (2016)[164]						Rimonabant, AM251	Glutamatergic transmission	Reverted ethanol-induced inhibition
Henricks et al. (2017)[146]	Acute (1–4 days) withdrawal after 6 weeks chronic intermittent alcohol vapor	Tissue	Wistar rats	AEA CB_1, DAGL, MAGL gene expression	Reduction Reduction			
Varodayan et al. (2017)[115]	2–3 weeks CIE vapor for 14 h a day	Brain slice	Sprague-Dawley rats			WIN, AM251	Spontaneous GABA transmission	CIE reduced WIN- and AM251-mediated effect
Ventral tegmental area								
Harlan et al. (2018)[165]	3 weeks withdrawal from CIE vapor	Brain slice	C57BL6/J mice	sIPSC frequency	Reduced	WIN, AM251	eCB-mediated $GABA_A$ inhibition (evoked IPSCs)	Increase

Table 2. Chronic Ethanol Exposure, Withdrawal, and ECB System Interaction, by Brain Region (Continued)

Brain Region and Study	Ethanol Exposure	System	Species	Measure	Effect	Drug	Synaptic Activity	Effect
Striatum								
Cippitelli et al., (2005);[140] Ortiz et al. (2004)[141]	30-min daily sessions on a fixed ratio 1 schedule of reinforcement self-administration	Tissue	Wistar rats	CB_1 gene expression	Decrease			
Vinod et al. (2006)[136]	72 h ethanol vapor (10–16 mg/l)	Tissue	Swiss Webster mice	CB_1 density and activation	Decrease			
Vinod et al. (2006)[136]	72 h ethanol vapor (10–16 mg/l) + 24 h withdrawal	Tissue	Swiss Webster mice	CB_1 density and activation	Recovery			
Vinod et al. (2012)[147]	70 days of two-bottle choice (24 h access/day)	Tissue	sP rats	CB_1-mediated G protein coupling eCB	Reduction Increase	Rimonabant	CB_1-mediated G protein coupling	Reversed
DePoy et al. (2013)[167]	2 weeks intermittent ethanol (16 h/day for 4 days per week)	Brain slice	C57BL6/J mice	2-AG	Increase		CB_1-mediated long-term depression	Abolition
Cerebellum								
Vinod et al. (2006)[136]	72 h ethanol vapor (10–16 mg/l)	Tissue	Swiss Webster mice	CB_1 density and activation	Decrease			
Vinod et al. (2006)[136]	72 h ethanol vapor (10–16 mg/l) + 24 h withdrawal	Tissue	Swiss Webster mice	CB_1 density and activation	Recovery			
Basavarajappa et al. (1999);[138] Basavarajappa et al. (2000)[168]	72 h ethanol (100 mM)	Cultured cerebellar granular primary neurons and SK-N-SH (human cell line)	Sprague-Dawley rats	AEA, 2-AG synthesis	Increase	Rimonabant	Ethanol induced 2-AG synthesis	Inhibited

Table 2. Chronic Ethanol Exposure, Withdrawal, and ECB System Interaction, by Brain Region (Continued)

Brain Region and Study	Ethanol Exposure	System	Species	Measure	Effect	Drug	Synaptic Activity	Effect
Basavarajappa et al. (1999);[138] Basavarajappa et al. (2000)[168]	72 h ethanol (100–150 mM)	Cultured cerebellar granular primary neurons and SK-N-SH (human cell line)	Sprague-Dawley rats	NAPE-PLD activity	Increase			
Basavarajappa et al. (2003)[171]	72 h ethanol (100–150 mM)	Cultured cerebellar granular primary neurons	Sprague-Dawley rats	AEA transport	Decrease	Rimonabant	AEA transport	No change
				FAAH activity	Decrease			

Note: 2-AG, 2-arachidonoylglycerol; AEA, arachidonoylethanolamide (anandamide); CB_1, cannabinoid receptor 1; CIE, chronic intermittent ethanol; FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; $GABA_A$, gamma-aminobutyric acid type A receptor; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acyl phosphatidylethanolamine–specific phospholipase D; sIPSC, spontaneous inhibitory postsynaptic current; WIN, WIN 55,212-2.

the literature, some recurring limitations emerged from the available studies. Therefore, the following are suggested as potential and important avenues of future research to address this gap in knowledge: (1) an emphasis on the synaptic protein landscape and synaptic function related to eCB signaling and alcohol interactions; (2) a focus on brain region specificity, given that different alterations in the eCB system are observed with alcohol exposure depending on brain region; (3) more consistent alcohol administration methodologies to control for differences in the eCB system that appear to be sensitive to different alcohol administration paradigms; (4) more research on the role that eCB signaling plays in alcohol withdrawal, particularly because very few studies have addressed this in terms of synaptic function; and (5) more research to address the lack of information concerning female animals and sex-specific differences as well as age-related effects.

Understanding the underlying mechanisms of alcohol and cannabinoid interaction in the different brain regions affected by AUD is still ongoing. Elucidating the role played by the eCB system in the alterations that occur in neural signaling and synaptic function after ethanol exposure and withdrawal may provide targets for developing pharmacotherapies for AUD. Additional mechanistic and physiological studies are needed to better understand how perturbations of the brain's eCB system may contribute to development of AUD.

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The Convergent Neuroscience of Affective Pain and Substance Use Disorder

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Opioids and alcohol are widely used to relieve pain, with their analgesic efficacy stemming from rapid actions on both spinal and supraspinal nociceptive centers. As an extension of these relationships, both substances can be misused in attempts to manage negative affective symptoms stemming from chronic pain. Moreover, excessive use of opioids or alcohol facilitates the development of substance use disorder (SUD) as well as hyperalgesia, or enhanced pain sensitivity. Shared neurobiological mechanisms that promote hyperalgesia development in the context of SUD represent viable candidates for therapeutic intervention, with the ideal strategy capable of reducing both excessive substance use as well as pain symptoms simultaneously. Neurocognitive symptoms associated with SUD, ranging from poor risk management to the affective dimension of pain, are likely mediated by altered activities of key anatomical elements that modulate executive and interoceptive functions, including contributions from key frontocortical regions. To aid future discoveries, novel and translationally valid animal models of chronic pain and SUD remain under intense development and continued refinement. With these tools, future research strategies targeting severe SUD should focus on the common neurobiology between negative reinforcement and affective elements of pain, possibly by reducing excessive stress hormone and neurotransmitter activity within shared circuitry.

Keywords: alcohol; cingulate cortex; insula; opioids; pain; reinforcement

A central feature of substance use disorder (SUD) is the emergence of negative affective or emotional states that influence the motivational properties of misused substances.[1] Individual propensity to experience pain-related negative affect, for example, is hypothesized to be associated with the maintenance of both opioid use disorder (OUD) and alcohol use disorder (AUD). Chronic pain is estimated to affect approximately 20% of adults worldwide,[2] a number that will likely increase over

the next several decades given the aging global population. Accordingly, opioids and/or alcohol may be sought and taken in excessive amounts to alleviate such symptoms.[3,4] From a neuroanatomical perspective, ascending nociceptive circuitry is well known to interact with and alter the function of frontocortical reinforcement systems key to the development and maintenance of both OUD and AUD.[5] The current state of neuroscience research aims not only to understand how these interactions manifest in the brain, but also to exploit these discoveries to promote novel therapeutic strategies targeting both chronic pain and SUD.[6]

This review focuses on two widely used analgesic agents, opioids and alcohol. Excessive use of either substance generates neuroadaptations that likely contribute to negative reinforcement processes in which efforts to achieve pain relief intersect with the likelihood of developing SUD, sometimes known as SUD liability.[7] Historically, the majority of preclinical pain studies have focused on peripheral and spinal nociceptive processes, yet have produced few translational therapies for chronic pain or safe alternatives to opioid-based analgesia.[8] Although alcohol represents another widely utilized strategy for pain relief,[9] the many pathophysiological risks associated with heavy drinking considerably outweigh the analgesic benefits.[10]

The most recent conceptualizations and research efforts have attempted to understand the specific contributions of pain-associated negative affect to the establishment of a variety of SUD. These efforts have focused on the role of central nociceptive and motivational brain areas underlying the transition to chronic pain and its potentially crucial relationship to SUD.[11,12] From a neurobiological perspective, this review describes key contributions from frontocortical areas that represent a shared neuroanatomical substrate for the intersection of pain and SUD-related symptomatology. Although this review focuses on opioids and alcohol, it is important to note that other misused substances—including nicotine and cannabis—can act as analgesics, and integrative mechanisms described in this review may play a role in the manifestation of one or more types of SUD.

PAIN RELIEF AS NEGATIVE REINFORCEMENT IN SUD

Opioid analgesics are the most powerful and effective medications for the treatment of acute pain.[13] Opioids are also widely accepted for use with intractable pain related to cancer or end-of-life care. Both naturally occurring (e.g., morphine) and synthetic (e.g., fentanyl) opioids produce strong and quantifiable analgesia across multiple modalities in both humans and animal models. The opioid receptors (mu, kappa, and delta) differ by the endogenous ligands that bind to them and by the range of effects the receptors produce, which is largely dependent on receptor location.[14] The pain-relieving properties of opioids are predominately mediated by mu-opioid receptor function based on the high binding affinity of opioid analgesics to mu-opioid receptors; however, activities at both kappa- and delta-opioid receptors also mediate analgesia.[14,15] Opioid analgesics also can produce euphoria and reduce negative emotional states (e.g., stress, anxiety, depression), which is attributed to the high density of opioid receptors across limbic brain regions.[16] There is well-described evidence that acute alcohol administration also produces analgesia in both humans and animals, but to a lesser degree than opioids.[6] From a neuropharmacological perspective, alcohol analgesia relies on the engagement of endogenous opioid signaling,[17] but also involves additional mechanisms including G protein-activated inwardly rectifying potassium (GIRK) channel activity.[18] A meta-analysis by Thompson and colleagues found a strong linear relationship between alcohol consumption and reported analgesia in humans.[19] However, some limitations of the Thompson review should be noted, including its reliance on a limited number of studies (mostly in men) where effect sizes were collapsed across several pain modalities (thermal

and mechanical). Moreover, no patient groups were included in the reviewed studies, highlighting the urgent need for additional work in this clinical area. Analgesia was reported to be strongest with alcohol levels that exceed the National Institute on Alcohol Abuse and Alcoholism (NIAAA) definition of binge drinking.[20] This identifies the potential risk involved in consuming alcohol for analgesic purposes.[21] Furthermore, authors from an empirical study examining the interaction of pain and alcohol-induced analgesia found that hazardous drinkers (determined by AUDIT-C scores) had a greater urge and intention to drink alcohol when given experimentally induced pain compared to hazardous drinkers without pain induction.[22] This highlights an important motivational aspect of drug-induced analgesia, where acute pain can increase the desire to drink alcohol or take opioids as an active strategy for reducing pain and associated negative emotional states. For this reason, opioids and alcohol often may be used by some individuals for a combination of pain management and stress relief.

In contrast to acute pain treatment, there is limited evidence of the utility of opioid treatment for most chronic pain conditions aside from cancer pain or pain during end-of-life care.[23] There are also serious safety concerns that need to be considered when prescribing opioids for chronic pain, including risk of developing OUD as well as acute overdose and death; for more information, see the Centers for Disease Control and Prevention's guideline for prescribing opioids for chronic pain.[23] With regard to alcohol, Zale and colleagues describe a curvilinear association between drinking and pain outcomes.[24] Whereas low to moderate alcohol use is associated with analgesia, excessive drinking is associated with poorer pain trajectories over time.[24] Low to moderate drinking was defined as drinking below government cutoffs for hazardous or excessive drinking, while excessive drinking was defined as either binge (> 4 drinks in 2 hours for women; > 5 drinks in 2 hours for men) or heavy drinking (number of drinks on any day or per week; for women, > 3 and > 7, respectively; for men, > 4 and > 14, respectively).[24] As mentioned above, alcohol is an effective analgesic over a dose range that overlaps the NIAAA definition of "at-risk" or binge drinking limit (females, ≥ 4 drinks, and males, ≥ 5 drinks, in about 2 hours; https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/binge-drinking).[19] If individuals limit their drinking below this point, they may achieve some analgesic efficacy with a reduced risk of later poor health outcomes. However, if they cross this line (perhaps to achieve greater analgesia), it places them at risk of eventually developing AUD and emerging hyperalgesia symptoms.

One key reason for the increasing use of opioids and alcohol for pain relief is the development of analgesic tolerance with repeated and/or extensive use. Tolerance refers to the principle that higher dose amounts of a substance are necessary to maintain the same biochemical and perceptual effects over time,[25] which both complicates treatment regimens and heightens SUD risk. A small prospective clinical study examined the effects of short-term opioid use on analgesic tolerance and pain sensitivity in the context of chronic pain.[26] Thermal pain thresholds and pain tolerance were assessed in individuals with chronic lower back pain, both before and after 1 month of an escalating oral morphine treatment regimen. A short-acting opioid was given prior to pain testing to examine changes to the analgesic efficacy of opioids following this 1 month of morphine treatment. Under this state, there was a significant decrease in pain thresholds and tolerance on the cold pressor test (measure of cold pain sensitivity), but no effects on heat-related pain. The rapid development of analgesic tolerance to opioids adds support to the limited clinical effectiveness of using opioids for long-term pain treatment. Tolerance to the analgesic and euphoric effects of opioids develops faster than tolerance to other physiological symptoms, including respiratory depression.[27] This explains why the risk of respiratory depression increases with escalated opioid use or in those who formerly misused opioids heavily and renewed opioid use after a period of protracted abstinence.

The development of analgesic tolerance following chronic alcohol exposure also has been well described in animal research,[6,17,28] but there is a lack of empirical human trials investigating the impact of tolerance on alcohol's analgesic effects.[24] Also unknown is how analgesic tolerance promotes alcohol craving or escalation of alcohol use in attempts to maintain analgesic effects over time.

Excessive use of alcohol and/or opioids may lead to states where both analgesic tolerance and hyperalgesia symptoms coincide.[29] Hyperalgesia is a form of pronociceptive system sensitization that behaviorally manifests as heightened pain sensitivity. Analgesic tolerance, along with the consequent escalation of analgesic use, contributes to the development of hyperalgesia, and are all hallmarks of opioid and alcohol dependence. In an opioid- and alcohol-dependent state, abstinence results in somatic withdrawal signs, pain, negative affect, and drug craving. These negative consequences can drive escalation of use over time, where negative reinforcement is the primary motivator for continued use or renewal during relapse.[1] Carcoba and colleagues examined the role of negative affect in opioid withdrawal-induced hyperalgesia in heroin-dependent individuals.[30] Compared to healthy controls, individuals in acute withdrawal (24 to 72 hours) and those in protracted withdrawal (~ 30 months) from heroin exhibited decreased pain thresholds and tolerance during an ischemic pain procedure. These hyperalgesic effects were heightened by viewing negative pictures (International Affective Picture System) beforehand, which elicit negative emotional states. Opioid-enhanced pain sensitivity can also play a role in cue-induced opioid craving following protracted abstinence. In another study, the cold pressor test was used to examine pain responses in abstinent individuals with a history of OUD.[31] These individuals had shorter periods of pain tolerance and reported higher ratings of pain-related distress compared to healthy controls. There was also a positive association between pain-related distress and opioid craving. In a cross-sectional study, individuals undergoing medication-assisted treatment (MAT) with methadone or buprenorphine were examined for opioid craving and recent illicit opioid use.[32] The investigators found that chronic pain was present in 68% of the sample and was associated with threefold higher odds of reporting craving, potentially placing this population at greater risk of relapse. Similarly, in a separate study, chronic pain levels at baseline were correlated with lower pain tolerance, greater stress reactivity during a cold pressor task, and posttest levels of opioid craving in individuals with comorbid pain and OUD.[33] Within comorbid pain and OUD groups, individuals who currently or formerly used MAT for OUD demonstrated increases in stress-reactivity to pain compared to opioid-naïve individuals with chronic pain. Furthermore, abstinent individuals who formerly used MAT for OUD demonstrated increased stress-reactivity to pain for some measures compared to current MAT users, indicating long-lasting consequences of OUD on neurophysiological outcomes.

Similar to opioids, hyperalgesia induced by alcohol withdrawal contributes to alcohol misuse and the development of AUD.[6] There are strong associations between alcohol consumption, pain, and pain-related disability.[34,35] In a secondary analysis of two clinical trials, Witkiewitz and colleagues found that greater pain scores were associated with alcohol drinking and increases in negative affect 1 year after treatment for AUD.[36] Using another large clinical data set, Yeung and colleagues examined the relationship between alcohol and pain interference (i.e., how pain interferes with everyday life).[35] In this analysis, higher alcohol consumption at baseline was associated with lower pain interference at 1-year follow-up. However, the opposite was true for individuals who exhibited more AUD symptoms. For them, higher baseline alcohol consumption was significantly related to higher pain interference at 1-year follow-up, indicating that the detrimental effects of alcohol on pain interference may emerge as the severity of the disease progresses. There is also a strong association between alcohol consumption, chronic pain, and pain-associated disability. Among persons with chronic pain, disabling pain was strongly associated with their

level of alcohol consumption.[37] There is some evidence that chronic pain status may be predictive of future drug and alcohol use. In prospective epidemiological studies, self-reported pain interference was predictive of AUD development,[38] and persistent pain was associated with increased odds of opioid use (adjusted odds ratio [*AOR*] = 5.4) and heavy alcohol use (*AOR* = 2.2) compared to no pain.[39]

With human research, it is very difficult to determine the direction of causality for the relationship between SUD and pain. Fortunately, a major benefit of animal research is the care with which experimental conditions can be controlled to determine the direction of causality for these complex associations. Preclinical animal research has been critical for the modeling of interactions between pain and SUD, and some of the most widely used techniques are described here.

ANIMAL MODELS TO EXAMINE PAIN AND SUD INTERACTIONS

Key symptomatology of OUD and AUD—including escalation of drug intake, compulsive drug seeking, development of hyperalgesia, and the emergence of negative affective states—can be reliably modeled in rodents. When discussing drug-induced hyperalgesia, it is necessary to discriminate that nociception and pain are different phenomena. Nociception refers to the neural process of encoding noxious stimuli, whereas pain refers to a personal experience that is influenced by biological, psychological, and social factors. Pain is therefore a subjective and inherently emotional experience. Accordingly, the empirical assessment of pain in rodents can be challenging. It is possible, however, to assess nociception and affective pain-like behavior in rodents through a variety of assays. Preclinical animal models also provide valuable tools for investigating the somatic and behavioral symptoms of SUD, identifying neurobiological changes associated with SUD, and testing medications to alleviate symptoms of dependence and reduce abuse liability. These models impact medication development and increase understanding of the behaviors that contribute to the development of SUD. There are several different procedures for inducing opioid and alcohol dependence in animals. Most involve the general procedure of repeatedly putting animals through a period of intoxication where the drug is administered by the experimenter or self-administered by the animals. This is followed by a period where the drug is not available, which produces a state of spontaneous withdrawal. As this cycle of intoxication and withdrawal is repeated, animals will begin to exhibit symptoms of dependence, including escalation of intake (if the drug is self-administered), pain-like behavior, compulsive drug-seeking behavior, and the emergence of negative emotional states (e.g., anxiety-like behavior).[40] When the drug is administered by the experimenter, the behavioral and neurochemical consequences of drug escalation can be mimicked by giving animals an escalating dose regimen to achieve a state of dependence.[40] In rodents, the most commonly used routes of administration for opioids include intravenous self-administration and subcutaneous administration, while the routes of administration for alcohol include oral self-administration, ethanol vapor exposure, intragastric gavage, a liquid diet containing alcohol (e.g., Lieber-DeCarli diet), and intraperitoneal administration.

Measurement of Nociception and Affective Pain in Animals

There are numerous tests to assay pain-like behavior in rodent models of psychiatric disease,[41] although the most common tests of nociceptive behavior in the context of hyperalgesia include von Frey[42] and Hargreaves[43] tests of mechanical hypersensitivity and thermal hypersensitivity, respectively. These reflexive-based tests involve applying a mechanical or thermal stimulus to the rodent's hind paw and measuring either the paw withdrawal threshold (typically in grams of pressure) for a graded mechanical stimulus or the paw withdrawal latency (typically in seconds)

for a constant thermal stimulus. A higher paw withdrawal threshold or latency compared to baseline is associated with an analgesic or anti-nociceptive process (e.g., following administration of an opioid substance), while a lower paw withdrawal threshold or latency is associated with hyperalgesia (i.e., more sensitive to the stimulus when compared to baseline). As discussed earlier, the subjective pain experience can greatly impact motivational processes associated with the transition to SUD. One shortcoming of these reflexive-based assays is the inability to assess the motivational and affective dimensions of pain, which are hypothesized to influence the transition to both chronic pain states[44] and SUD.[45,46] Neuroscientists are beginning to employ additional behavioral tests that attempt to more closely assess the cognitive and motivational aspects of pain-like behavior beyond the somatic or sensory components. These non-reflexive-based assays allow the potential to examine the contribution of negative affective-like states towards activity avoidance and pain interference in the context of SUD.[47,48] In the mechanical conflict-avoidance system (MCS) task, animals traverse mechanically noxious probes of varying heights to avoid a bright aversive light, escaping to reach a goal chamber that is dark. A longer latency to exit onto the probes reflects increased pain avoidance-like behavior as a motivational correlate of hyperalgesia. The specific strengths and limitations of the MCS procedure have been described, illustrating its utility in measuring both analgesic and hyperalgesic conditions.[47,49,50] Another innovative technique in this area is the Orofacial Pain Assessment Device (OPAD), which pairs a thermal stimulus conflict with access to an appetitive reward[51] and can be readily applied to oral alcohol or opioid self-administration. These reflex-based and non-reflex-based pain assays can be used in tandem to more comprehensively examine the effects of opioid and alcohol dependence on both somatic and affective pain-like behaviors in rodents.

Measurement of Opioid-Induced Hyperalgesia in Animals

Induction of opioid dependence in rodents can be achieved through intravenous self-administration where animals are given extended (or long) access (LgA; 6 hr, 12 hr, or 24 hr) versus limited (or short) access (ShA, 1 hr) to opioids,[52] including prescription opioids such as fentanyl and oxycodone.[53] In this model, LgA animals exhibit hallmarks of OUD including escalation of opioid intake, compulsive opioid seeking, development of hyperalgesia, and the emergence of negative emotional states. Male Wistar rats given LgA (12 hr) to heroin self-administration (0.06 mg/kg/infusion) exhibit decreased paw withdrawal thresholds compared to ShA (1 hr) animals during spontaneous withdrawal, indicating opioid-induced mechanical hyperalgesia.[54] Interestingly, the emergence of opioid-induced hyperalgesia coincided with escalated heroin intake in LgA animals, which was not observed in ShA animals.[54] In this study, increased heroin intake was significantly correlated with increased pain-like behavior (lower paw withdrawal thresholds), demonstrating the close relationship between opioid intake and pain symptoms in the context of dependence. Repeated subcutaneous administration of opioids can also induce dependence and pain-like behavior in rodents. Rats given repeated subcutaneous doses of heroin for 5 days exhibited decreased paw withdrawal thresholds compared to animals given a single dose of heroin, demonstrating the ability of opioids to drive nociceptive system sensitization.[29] In a separate study, male Wistar rats were given an escalating dose regimen of morphine (10 mg/kg to 20 mg/kg) over 2 weeks to examine the effects of morphine dependence on the sensory and motivational/affective components of pain-like behavior, using von Frey and MCS procedures, respectively.[49] Opioid-dependent animals exhibited an increased latency to exit onto a bed of noxious mechanical probes during withdrawal compared to saline-injected controls, indicating increased pain-like

avoidance with escalated morphine use. There was a modest but significant correlation between changes in mechanical hypersensitivity and pain-like avoidance behavior, indicating that the von Frey and MCS procedures examine overlapping, but not identical, measures of pain-like behavior. Continued investigations that shed light on individual differences in opioid and pain sensitivity along both somatic and affective dimensions also may help researchers to maximize the beneficial use of opioid analgesics while minimizing OUD liability.

Measurement of Alcohol-Induced Hyperalgesia in Animals

The somatic and affective symptoms of AUD can be reliably modeled in rodents using chronic intermittent ethanol vapor (CIEV) exposure.[55] The intermittent procedure involves daily cycles of alcohol vapor (producing peak blood alcohol levels of 150–200 mg/dl) and alcohol withdrawal. After several weeks of CIEV, alcohol-dependent male Wistar rats exhibited decreases in paw withdrawal thresholds during spontaneous withdrawal compared to non-dependent controls, indicating alcohol-induced mechanical hyperalgesia.[54] In a separate study, 4 weeks of CIEV produced thermal hyperalgesia in alcohol-dependent male Wistar rats compared to nondependent controls.[56] This increase in pain-like behavior was attenuated following either alcohol administration by the experimenter or alcohol self-administration. The anti-hyperalgesic effects of acute alcohol treatment in alcohol dependence provides strong evidence of the motivation to drink alcohol to ameliorate withdrawal symptoms and decrease pain. In a nonforced contingent ethanol vapor self-administration study, male Wistar rats were allowed to nose poke for ethanol vapor (8 hr/day) over either 8 or 24 sessions, which produced nonescalated and escalated nose poking for ethanol vapor exposure, respectively.[57] Like the previous CIEV studies, rodents who escalated nose pokes demonstrated decreased paw withdrawal thresholds during withdrawal compared to nonescalated animals, indicating increased pain-like behavior. Additional models of alcohol dependence, including chronic intermittent two-bottle choice and the Lieber–DeCarli diet, produced mechanical and thermal hyperalgesia in male Sprague Dawley rats,[58,59] and the "Drinking in the Dark" procedure facilitated hyperalgesia in female and male C57BL/6J mice.[60]

Examining How Pain Influences Opioid and Alcohol Use in Animals

Another interesting area of preclinical pain research involves examining the effects of persistent pain on drug abuse liability. Neuropathic pain, fibromyalgia, low back pain, and osteoarthritis are common medical conditions that contribute to the burden of chronic pain disorders. Accordingly, preclinical models of neuropathic pain (e.g., spared nerve injury, spinal nerve ligation) and inflammatory pain (e.g., complete Freund's adjuvant [CFA]) are frequently used to examine the effects of chronic pain on behavior and neurochemistry in rodents. Martin and colleagues found that, compared to controls, nerve-injured male Fisher 344 rats required higher amounts of heroin to maintain heroin self-administration and were more sensitive to mu-opioid receptor antagonist-induced increases in heroin self-administration.[61] In a study examining how persistent inflammatory pain alters morphine preference, CFA reduced the number of morphine conditioning sessions required to acquire morphine-conditioned place preference in male Wistar rats.[62] Hipólito and colleagues found that CFA altered heroin self-administration in a dose-dependent manner in male Sprague Dawley rats.[63] High unit doses (0.2 mg/kg/infusion) were more reinforcing, and low unit doses (0.05 mg/kg/infusion) were less reinforcing. These preclinical examinations provide evidence for the hypothesis that the driving force for motivation to self-administer opioids in individuals with an underlying pain condition may be in part to seek relief from chronic pain. These findings may also indicate that shared neural substrates promote both substance use and pain chronification, or the process by which acute pain becomes chronic, as discussed in the next section.

A number of additional studies have examined the effects of chronic pain on alcohol consumption

in rodents.[64] Sciatic nerve–injured CD1 male mice consumed more alcohol (20% ethanol) and exhibited increased anxiety-like behavior compared to sham-operated mice, suggesting that a chronic pain state drives increased alcohol consumption.[65] In a mouse model of osteoarthritis, male C57BL/6J mice consumed significantly more alcohol than sham controls during a two-bottle choice test of escalating alcohol concentrations (2.5% to 20%).[66] During a 20% ethanol continuous access test, CFA increased alcohol drinking in male C57BL/6J mice, but did not increase drinking in female C57BL/6J mice.[67] In contrast to these findings in mice, a recent study found no effect of CFA on alcohol self-consumption or alcohol preference in male Wistar rats.[68] However, this study discovered that the relationship between alcohol drinking levels and hyperalgesia symptoms reversed between acute (1-week) and chronic (3-week) periods post-CFA administration, suggesting that either the motivational or analgesic effects of alcohol may be altered over the time course of chronic pain.

Altogether, there appear to be clear effects of chronic pain on opioid intake, motivation for opioids, alcohol consumption, and alcohol preference that are largely dependent on factors including rodent species and sex. In summary, repeated and extensive exposure to opioids and alcohol promotes escalation of intake and pain-like behavior, which are sequelae that can in turn exacerbate abuse liability and SUD disease severity.

SHARED FRONTOCORTICAL SUBSTRATES FOR AFFECTIVE PAIN AND SUD

In addition to somatosensory elements, both affective/emotional and cognitive/motivational dimensions can augment pain-related morbidity.[6] Chronic pain can generate continual negative affective states and promote new cognitive strategies and behaviors to avoid pain. Consequently, pain relief itself activates reward circuitry and is experienced as a positively valenced emotional state.[69] It is thus hypothesized that the emergence of painful states following chronic or excessive opioid or alcohol exposure facilitates negative reinforcement processes whereby individuals seek relief from pain by escalating use of these substances, culminating in the development of psychiatric sequelae including SUD.[45,46] Specific alterations in frontocortical activity may facilitate pain and promote maladaptive behaviors in close association with pain-related negative affective states. As such activity is heavily impacted by chronic or excessive opioid and alcohol exposure, further interrogation of within- and between-circuit neuroadaptations is warranted to better understand the pathological intersection of pain and SUD.[46,70]

INSULAR AND CINGULATE CORTICES AND AFFECTIVE PAIN PROCESSING

The insular cortex and the cingulate cortex represent key components of a distinct neural network within the larger executive control system of the prefrontal cortex. Communication within these areas is hypothesized to facilitate attribution of emotional salience to both internal and external stimuli, including pain-related noxious stimuli.[9] Of particular interest is the role of frontocortical regions in higher nociceptive processing, as well as their historical association with SUD.[5] Pain is a multidimensional experience, which comprises both sensory and affective-motivational components.[71] Through studies of these regions both in isolation and as a functional network, the insula and cingulate have been identified as key areas for supraspinal processing of the affective dimension.[18] Imaging studies have also identified heightened activity in the insula and cingulate with the anticipation of pain and have correlated perceived pain intensity with degree of concurrent activity in the insula and cingulate in human subjects.[72,73] In rodent models, selective lesions of the cingulate have been shown to reduce pain-related aversion without altering the sensory element of noxious stimuli.[74,75] The insula has reciprocal connections with the cingulate and receives nociceptive

information directly from the thalamus.[76] Moreover, insula connectivity with subcortical regions such as the amygdala may facilitate emotional arousal to noxious stimuli.[76,77]

Resting-state functional magnetic resonance imaging (fMRI) analyses have identified a precise network based in the insula and cingulate that extends to several subcortical regions referred to as the salience network. The salience network model was developed from the integration of multiple human fMRI studies that ultimately led to the hypothesis that this particular circuitry recognizes and assimilates interoceptive and external information, recruits and derecruits additional executive networks to engage the appropriate cognitive processes (focusing attention to stimuli, including noxious stimuli), and ultimately regulates an adaptive behavioral response.[78] Alterations in the salience network are observed in individuals with chronic pain and are associated specifically with greater pain catastrophizing,[79] a phenomenon that is believed to be closely related to the chronification of pain. The network has most commonly been investigated in human and nonhuman primate models, but was recently confirmed in rodents, validating crucial contributions from the insula and cingulate cortex.[80]

DYSREGULATION OF THE SALIENCE NETWORK BY ALCOHOL AND OPIOIDS

Research has provided evidence that AUD dysregulates activity of the insula-cingulate salience network in humans, typically indicated by fMRI analyses. This alteration is believed to impair executive function, compromising the ability to make appropriate or cognitively demanding decisions.[81] Salience network deficits may specifically contribute to the maintenance or exacerbation of AUD by making an individual unable to clearly discern risky behaviors, such as the decision to seek out and consume excessive amounts of alcohol despite adverse consequences. This network may be particularly vulnerable in AUD patients exposed to stressful conditions due to cingulate dysfunction.[82] Investigators have also found that excessive drinking may disrupt normal associations between interoception and pain.[83] A similar involvement of endogenous opioid signaling in salience network function is well known.[84] Alterations in the network's connectivity are related to resting state dysfunction[85] as well as to relapse behaviors[86] in patients with OUD. More studies are needed to examine salience network activity in populations with OUD in relation to hyperalgesia symptoms, especially because pain symptoms can promote opioid craving even after months of abstinence.[31]

Although the salience network is most commonly examined in humans, several preclinical animal studies have begun to examine the importance of this construct with relation to pain and alcohol exposure. Interestingly, in mice, the insula and cingulate were discovered to have a role in the social transfer of pain associated with hyperalgesia following alcohol withdrawal.[87] Another recent study found several interbrain regional correlations of glucocorticoid receptor (GR) phosphorylation in animals experiencing a binge alcohol withdrawal episode in the context of chronic inflammatory pain.[68] The insular cortex acted as a hub for these correlations with other nociceptive regions investigated (including the cingulate cortex and central amygdala), suggesting coordinated activity in insula circuitry and glucocorticoid signaling in the context of pain and alcohol withdrawal. This type of within-subject molecular analysis at the animal level may model human fMRI analyses of related network activity. These circuit-based relationships also have been hypothesized to play a key role in the motivational processes relevant to SUD.[5] Finally, a recent conceptual review postulated that neurovisceral feedback and interoceptive dysregulation by opioids and alcohol can be traced to alterations in gut microbiota,[88] highlighting the need for further investigation of the gut-brain axis in SUD and related pain.

BRAIN STRESS SIGNALING IN AFFECTIVE PAIN AND SUD

Given that chronic and unmitigated pain represents a significant stressor, elucidation of chronic opioid- and alcohol-induced neuroadaptations within brain stress systems may provide valuable insights into potential mechanisms underlying the transition to SUD in vulnerable individuals. Indeed, the role of central stress hormone and neuropeptide signaling in response to stress has emerged as a conceptual bridge between chronic substance use, affective and cognitive disruption, and propensity to relapse.[89] As the key integrative link between the systemic and central brain stress response, the hypothalamic-pituitary-adrenal (HPA) axis is responsible for orchestrating adaptive processes that return an organism to homeostasis following exposure to a stressor. Release of corticotropin-releasing factor (CRF) from the hypothalamus initiates this process by regulating the production and processing of pro-opiomelanocortin from the anterior pituitary. The pro-opiomelanocortin transcript produces two key peptides related to the effective management of both stress (adrenocorticotropic hormone) and pain (beta-endorphin), illustrating the close relationships between these two vital physiological systems. Adrenocorticotropic hormone acts to facilitate the production and release of glucocorticoids from the adrenal cortex, after which the systemic response is under the control of critical negative and positive feedback mechanisms, whereby glucocorticoids can inhibit or stimulate (respectively) their own genomic and nongenomic actions by binding to GRs in the brain.[90] Stress sensitization via potentiated GR signaling may represent one mechanism for intensification of SUD-associated negative affective symptoms, termed hyperkatifeia.[46]

Alcohol-dependent animals display a functional increase in brain GR signaling that appears to emerge during the transition to dependence.[91] GR antagonism reduces escalated drinking in both preclinical animal models and in individuals suffering from AUD.[92] It is also interesting that systemic administration of the GR antagonist mifepristone alleviates mechanical hyperalgesia symptoms observed in animals fed an alcohol diet.[93] These convergent findings suggest that targeting excessive stress signaling may be capable of treating both excessive drinking and pain symptoms in the context of AUD. Less is understood about these associations in relation to OUD, although similar relationships connecting negative reinforcement processes to pain and OUD have been proposed.[94,95] These conceptualizations are supported by research indicating links between serum cortisol levels and opioid withdrawal in humans[96] and functional activation of negative reinforcement brain centers in opioid-dependent animals.[97] Although systemic CRF_1 receptor antagonism has been shown to alleviate hyperalgesia symptoms in opioid-dependent animals,[54] no studies have investigated the potential contribution of GR signaling in this process. Given the role of chronic stress and glucocorticoid activity in exacerbating pain,[98] additional work is necessary to determine the relationships between stress hormone signaling and pain symptoms in patients suffering from AUD and OUD.

CONCLUSIONS

Few effective therapies exist for SUD or chronic pain. The accretive pathophysiology and shared neurobiological interactions of these disease states likely complicate their effective treatment. Powerful reinforcement processes maintain the use of opioids and alcohol to manage pain as well as the negative affective states that underlie chronic pain experiences. Future translational research priorities should aim to bridge gaps in our understanding of how opioids and alcohol act on nociceptive and higher motivational circuitry to drive tolerance and hyperalgesia symptoms that may exacerbate SUD. Numerous symptoms are regularly associated with severe SUD, ranging from poor risk management to the cognitive/affective dimension of pain. These symptoms are likely driven by neuroadaptations within key anatomical elements that regulate higher

executive functions, including key contributions from the cingulate and insula cortices.

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Forebrain-Midbrain Circuits and Peptides Involved in Hyperalgesia After Chronic Alcohol Exposure

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People living with pain report drinking alcohol to relieve pain. Acute alcohol use reduces pain, and chronic alcohol use facilitates the emergence or exaggeration of pain. Recently, funding agencies and neuroscientists involved in basic research have turned their attention to understanding the neurobiological mechanisms that underlie pain-alcohol interactions, with a focus on circuit and molecular mediators of alcohol-induced changes in pain-related behavior. This review briefly discusses some examples of work being done in this area, with a focus on reciprocal projections between the midbrain and extended amygdala, as well as some neurochemical mediators of pain-related phenotypes after alcohol exposure. Finally, as more work accumulates on this topic, the authors highlight the need for the neuroscience field to carefully consider sex and age in the design and analysis of pain-alcohol interaction experiments.

KEYWORDS: alcohol; pain; withdrawal; dependence; hyperalgesia; allodynia

Chronic pain increases the risk for development of alcohol use disorder (AUD). Given that acute alcohol consumption can reduce pain, humans sometimes drink alcohol for relief of pain. Chronic alcohol consumption, however, can increase pain sensitivity during withdrawal and facilitate pain sensitization related to comorbid pain conditions.[1] Ascending and descending nociceptive circuitry

and higher order pain processing centers exhibit a high degree of overlap with the brain circuits that mediate behaviors associated with alcohol reinforcement and with AUD, alcohol dependence and withdrawal.[1] Recently, the National Institute on Alcohol Abuse and Alcoholism has prioritized research that aims to uncover the neurobiological mechanisms that underlie pain-associated increases in alcohol drinking (i.e., self-medication) and the emergence or exaggeration of pain phenotypes by chronic alcohol exposure. Layered into this research topic are questions of sex differences and species differences, age at time of alcohol exposure and testing, and potential effects of dose, duration, route, and pattern of alcohol exposure. In response to this programmatic shift, neuroscientists involved in basic research have increased their investigation of the neurobiological mechanisms underlying these phenomena, including circuit interrogation and testing the role of neuropeptides, which are the two focal points of this review. Discussed below are the roles of two circuits between the limbic forebrain and the midbrain, more specifically, projections descending from the central amygdala (CeA) to the periaqueductal gray (PAG) and projections ascending from the PAG to the bed nucleus of the stria terminalis (BNST), as well as peptidergic transmission within those regions and circuits, in mediating alcohol-pain interactions. Although each of these brain regions can be subdivided, the literature in this area is in its nascent stages and in many cases the contributions of subregions have not been examined; therefore, this review's language regarding brain regions reflects the resolution provided within the cited studies.

SUPRASPINAL CIRCUITS IN ALCOHOL WITHDRAWAL HYPERALGESIA

In the brain, the molecular and cellular mechanisms involved in pain processing are complex and diverse. The ventrolateral PAG (vlPAG) receives information from ascending pain pathways and is a major source of descending outputs responsible for inhibitory control of pain.[2,3] In adult male and female Sprague-Dawley rats, the analgesic and antihyperalgesic effects of opioid receptor agonists are in part attributed to their action in the PAG.[4] More specifically, opioid drugs can reduce pain in adult male Sprague-Dawley rats by facilitating the activity of descending vlPAG outputs to the rostral ventrolateral medulla (RVM),[5,6] and evidence suggests that endogenous opioids may modulate pain similarly in rodents.[7] In adolescent male Swiss Webster mice, mu opioid receptor activation also disinhibits tyrosine hydroxylase (TH)-positive (i.e., dopaminergic [DAergic]) neurons in the PAG via local gamma-aminobutyric acid-ergic (GABAergic) inputs.[8] Therefore, the PAG receives input from ascending pain modulation pathways, has reciprocal connections with the limbic forebrain, and mediates the analgesic effects of opioid drugs via multiple mechanisms and circuits. Furthermore, chronic alcohol exposure produces plasticity in the CeA[9] and BNST,[10] both of which are inputs to the PAG,[11,12] suggesting that the same circuitry responsible for pain modulation may be sensitive to prolonged alcohol use.

A majority of prior work examining the neurobiology of alcohol has focused on the investigation of individual brain regions, but progressively more attention is being paid to the acute and chronic effects of alcohol on brain circuits involved in nociception. The ultimate goal of circuit-level analysis of alcohol-related hyperalgesia is to facilitate the identification of potential treatment targets in humans with AUD living with pain. This may be achieved by establishing the molecular signature of cells that modulate pain and nociception via projections to other brain regions. For example, if specific receptor subtypes are preferentially enriched on a subset of projection neurons, then pharmacological modulation of those receptors may present a unique opportunity to modulate that circuit for reducing pain-related outcomes with minimal off-target effects.

VLPAG/DR TO BNST: AN ASCENDING ANTINOCICEPTIVE CIRCUIT

Dopamine (DA) neurons in the vlPAG/dorsal raphe (DR) were first characterized in a series of neuroanatomical studies, where they were reported to be a dorso-caudal extension of the ventral tegmental area (VTA).[13,14] Dopamine neurons in the vlPAG/DR are disinhibited by mu opioid receptor agonists and have roles in mediating pain and arousal in rats and mice of various strains and ages.[8,14–22] Notably, the vlPAG/DR and VTA project to the extended amygdala, where co-release of DA and glutamate activates neurons in the BNST and the CeA of rats and mice.[8,23-24] Therefore, it is reasonable to postulate that DA signaling in these extended-amygdala structures alters some aspects of the pain experience.

In support of this notion, bath application of alcohol promotes firing of vlPAG/DR DA neurons in brain slices taken from adolescent male Swiss Webster mice, potentially via modulation of glutamatergic transmission.[25] Furthermore, systemic administration via intraperitoneal injection of alcohol or morphine can increase extracellular DA levels in the BNST of presumably adult (230–250 g) male Sprague-Dawley rats,[26] and morphine increases phosphorylation of extracellular signal–regulated kinase (ERK) via dopamine D1 receptors in adult male and female C57 mice,[27] suggesting that drugs of misuse may modulate pain via activation of DA inputs to the BNST. Given the role of the BNST in regulating emotional and motivational behaviors, vlPAG/DR DAergic projections to the BNST may mediate or mitigate the affective aspects of chronic pain. Interestingly, both chemogenetic activation of vlPAG/DR DAergic neurons and optogenetic activation of vlPAG/DR TH-positive outputs to the BNST are antinociceptive in adolescent[8] and adult[20,28] male mice. This antinociceptive effect manifests as a reduction in basal pain sensitivity and attenuation of hypersensitivity after persistent intraplantar inflammation, demonstrating a possible role for DAergic projections from the vlPAG/DR to the BNST in pain-related outcomes.

THE VLPAG/DR-BNST CIRCUIT IN SEX-SPECIFIC MECHANISMS OF PAIN

In humans, pain drives greater functional connectivity between the PAG and limbic structures in men relative to women,[29] but the mechanisms behind these differences are unclear. Only a few molecular drivers of sex differences have been identified for pain,[30] with some evidence supporting a role for DA signaling in the vlPAG/DR and the BNST. In the midbrain, the vlPAG-RVM circuit is critical for mediating morphine antinociception and tolerance to this effect in adult male Sprague-Dawley rats;[4] the same effects are facilitated, at least in part, by morphine-microglia interactions in adult female Sprague-Dawley rats.[31] Chronic inflammatory pain increases presynaptic GABA release but decreases high-affinity tonic gamma-aminobutyric acid type A ($GABA_A$) receptor-mediated currents exclusively in vlPAG neurons of adolescent female Sprague-Dawley rats, an effect that may be associated with sex-specific morphine-induced antinociception, which was measured in adult Sprague-Dawley rats in the same study.[32] These data suggest that opioids and chronic inflammation alter GABAergic signaling in the vlPAG and influence pain sensitivity differently for males and females. Considering that the same vlPAG GABAergic neurons that regulate the activity of RVM-projecting cells also may govern the activity of vlPAG DAergic neurons in adolescent and adult mice,[8,22] it is possible that BNST-projecting vlPAG/DR DA-positive cells contribute to sex differences in pain processing. Previous work from the Kash lab and others have shown that DAergic cells in the vlPAG/DR robustly project to the BNST and that their activation reduces pain-related behaviors in adolescent and adult male

mice,[8,20] but these evaluations were performed only in male mice and did not examine circuit activity. Newly published data from the Kash lab indicate that optogenetic activation of DAergic inputs from the vlPAG/DR to the BNST alters nociception in adult male but not female C57 mice,[28] and that this activation is associated with subtle changes in dopamine receptor function assessed in brain slices. In contrast, local antagonism of DA D1 receptor in the BNST increases pain-like behavior only in adult female rats.[33] These data suggest not only sex differences, but also species differences, in the role of DAergic inputs from the vlPAG/DR to the BNST in mediating pain-related behavior.

DA REGULATION OF CRF FUNCTION IN THE BNST, SEX, AND REGULATION OF PAIN

The BNST is a center of integration for value representation, motivated behaviors, threat response, and drug use.[34] Although the potential role of the BNST in pain is not well characterized, it has been suggested that corticotropin-releasing factor (CRF) signaling in the BNST has a role in the sensory and affective-motivational components of pain,[35-41] which parallels data showing that CRF signaling in the CeA of adult male Sprague-Dawley rats facilitates pain-like responses via actions at CRF type-1 receptor (CRFR1).[42] Evidence for DA-CRF interactions comes from findings that DA enhancement of glutamatergic synaptic transmission in the BNST is regulated by CRFR1 activity in adolescent male C57 mice.[43] Although it has been assumed that this DA comes from the VTA, the vlPAG/DR remains an intriguing possibility as the source of DA in the BNST. Because DA neurons in the vlPAG/DR co-express vasoactive intestinal peptide (VIP) in mice[44] and VIP neurons in the vlPAG/DR terminate onto CRF neurons in the BNST, vlPAG/DR DA neurons may directly influence CRF signaling by innervating CRF neurons in the BNST. A more direct indication by Meloni et al. (2006)[45] shows that in adult male Sprague-Dawley rats, the majority of DA neurons innervating CRF neurons in the BNST originate in the vlPAG/DR. Therefore, vlPAG/DR DA neurons may interact with CRF signaling via direct cellular transmission onto CRF neurons in the BNST. Finally, BNST anatomy, CRF distribution,[46] DA modulation of pain[33] and behavioral effects[47] differ in male and female rats and mice. Therefore, it is possible that DA cells in the vlPAG/DR and CRF cells in the BNST work together to contribute to sex differences in pain. The Kash lab has begun to explore this possibility using in vivo imaging.[48] Briefly, the authors found that CRF neurons in the BNST are dynamically engaged during nociceptive processing; however, there is reduced activation of BNST CRF neurons during noxious heat exposure in adult female mice compared to males. It will be critically important to determine if the dynamics of CRF neurons in the BNST are altered following alcohol exposure. Although there has been more focus on the role of dopaminergic innervation of the BNST in pain-related outcomes, one study reported that activation of serotonergic projections from the DR to the CeA reduces negative affective behavior in adult male mice with chronic inflammatory or neuropathic pain.[49] This finding is especially intriguing given that selective serotonin reuptake inhibitors have been used to treat both AUD and pain disorders.

CEA TO VLPAG PROJECTIONS IN ALCOHOL WITHDRAWAL HYPERALGESIA

The vlPAG is densely innervated by descending inputs from the CeA.[50,51] In the context of chronic pain models, the CeA and its projections to the vlPAG have been tested for their role in pain-related behaviors in adult and adolescent rats and mice.[52-56] Early studies showed that electrical stimulation of the CeA in rodents produces analgesia, and that this effect is blocked by lidocaine-induced inactivation of PAG or by opioid receptor blockade in PAG, suggesting that cells

projecting from the CeA to the PAG modulate the nocifensive response in male Wistar rats weighing 140–160 grams.[57] Data from the Gilpin lab show that inactivation of the CeA as a whole (using tetrodotoxin),[58] or inactivation of cells projecting from the CeA to the vlPAG (using optogenetics),[11] is pronociceptive in adult male Wistar rats. Work from other groups shows that ERK activation in the amygdala (manipulations aimed at the CeA) is necessary and sufficient to induce lasting mechanical hypersensitivity in male Swiss Webster mice weighing 40–45 grams.[54] In recent years, it has become increasingly clear that the role of the CeA in mediating pain-like responses is affected by the CeA subregion (medial versus lateral division)[55] and CeA cell type (according to their morphology, electrophysiology and molecular signature)[52] being examined, as well as laterality of pain and amygdala,[53] and possibly also by chronic pain state, species, sex, and age of experimental subjects.

Based on this prior work, the Gilpin lab investigated the relationship between chronic exposure to high-dose alcohol, CeA-vlPAG circuit activity, and pain-related outcomes in rats. Adult male Wistar rats rendered alcohol-dependent via long-term alcohol vapor exposure (i.e., exposure models that produce physiological and behavioral signs of withdrawal upon termination of alcohol exposure) exhibit thermal hyperalgesia during withdrawal, which is not observed in rats that are nondependent alcohol drinkers or alcohol-naïve controls.[59] This effect is reversed by acute bolus systemic alcohol injections and by oral alcohol self-administration prior to nociception testing.[59] In subsequent work using optogenetic stimulation of CeA terminals in the vlPAG, alcohol-dependent adult male Wistar rats exhibited weaker connectivity between the CeA and the vlPAG during alcohol withdrawal, as evidenced by lower amplitude of inhibitory postsynaptic currents.[11] The authors also showed that pro-nociceptive manipulations in the CeA of rats can be reversed by mu opioid receptor blockade in the vlPAG (confirming the result found by Oliveira and Prado in 2001,[57] mentioned above), that optogenetic activation of CeA neurons projecting to the vlPAG attenuates hyperalgesia associated with alcohol withdrawal in alcohol-dependent rats, and that inhibition of these neurons produces thermal hyperalgesia in otherwise experimentally naïve adult male Wistar rats.[11]

AMYGDALAR MC4R SIGNALING IN ALCOHOL WITHDRAWAL HYPERALGESIA

Chronic alcohol exposure and withdrawal alters melanocortin 4 receptor (MC4R) expression in the CeA of adult male Wistar and Sprague-Dawley rats,[11,60] and site-specific antagonism of MC4Rs in the CeA reverses alcohol withdrawal hyperalgesia in adult male Wistar rats.[11] Antagonism of MC4Rs in the amygdala facilitates the antinociceptive effects of morphine and prevents the development of tolerance to the analgesic effects of morphine as well as the emergence of paradoxical hyperalgesia during morphine withdrawal in adult male Sprague-Dawley and Wistar rats.[61,62] Furthermore, MC4R antagonism reduces neuropathic pain in adult male Wistar rats.[63,64]

MC4Rs are expressed at most levels of the ascending and descending pain circuitry and induce plasticity by altering trafficking of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to the membrane.[65] Therefore, it would be of interest to know whether MC4R expression is enriched specifically in cells linking brain regions important for pain processing, that is, in pain circuits (e.g., on the postsynaptic membranes of vlPAG-projecting cells in the CeA). It is unclear whether MC4Rs are expressed or enriched on the postsynaptic membrane of vlPAG-projecting cells in the CeA. Ongoing work is examining whether this is the case and aims to test whether modulation of MC4R activity on vlPAG-projecting CeA cells attenuates alcohol withdrawal hyperalgesia and other pain states. Collectively, there is growing literature showing that MC4R antagonism in the CeA—and other sites in the central nervous system—reduces pain-related behavior in multiple

pain states. This is exciting when one considers that intranasal delivery of an MC4R antagonist blocks alcohol withdrawal hyperalgesia in adult male Wistar rats[59] and reduces anxiety-like behavior in male Sprague-Dawley rats weighing 150–160 grams,[66] suggesting that intranasal delivery of MC4R antagonists to treat pain conditions may hold promise for translation to the clinic.

AMYGDALAR CRF SIGNALING IN ALCOHOL WITHDRAWAL HYPERALGESIA

Many years of research have been devoted to understanding the behavioral effects of CRF-CRFR1 signaling in the CeA. Much of this work has focused on addiction-related behaviors, anxiety-like behavior, stress reactivity, fear-related behavior, and nociception in rats and mice.[9,56,67] CRF and CRFR1 messenger RNA (mRNA) and protein levels are highly expressed in the CeA of adult male Wistar rats,[68] CRF increases inhibitory transmission in the CeA, and this effect is altered by alcohol dependence in adolescent male Sprague-Dawley rats.[69] In the same study, chronic in vivo systemic CRFR1 antagonism during alcohol withdrawal prevented the emergence of dependence-like phenotypes during subsequent withdrawals in adult male Wistar rats.[69] Antagonism of CRFR1 in the CeA acutely reduces anxiety-like behavior in adult male Wistar rats,[70] reduces avoidant behaviors in adult male Wistar rats with high stress reactivity,[71] reduces escalation of alcohol drinking in alcohol-dependent and stressed adult male Wistar rats,[68,71] and attenuates hyperalgesia induced by nicotine dependence and predator odor stress in adult male Wistar rats.[58,72]

Recent work from the Gilpin lab shows that chronic alcohol exposure during adolescence leads to hyperalgesia and reductions in synaptic drive onto vlPAG-projecting CeA cells in rats, effects that last many weeks after termination of alcohol exposure in male but not female Wistar rats.[73] This latter finding is in agreement with prior work from the authors showing weaker CeA-vlPAG connectivity in alcohol-dependent adult male Wistar rats that are hyperalgesic during acute withdrawal.[11] It remains to be determined whether the effects of CRFR1 in the CeA on alcohol withdrawal hyperalgesia can be attributed to their expression on specific subsets of CeA projection cells. Ongoing work by the authors seeks to build on these initial circuit-level findings by determining (1) how the CeA-vlPAG circuit is modulated by the activity of specific peptide systems during alcohol withdrawal, and (2) the role of these cell type-specific circuits in mediating alcohol withdrawal hyperalgesia.

BIOLOGICAL FACTORS IN ALCOHOL WITHDRAWAL HYPERALGESIA

Men and women experience, process, and report pain differently.[74,75] Similarly, rodents exhibit sex differences in baseline nociception and responses to the antinociceptive effects of analgesic drugs.[76] Under healthy and chronic pain conditions, humans and non-human animals exhibit diffuse noxious inhibitory control (DNIC) of pain (also called inhibition of pain by pain) that is modulated by sex.[77,78] In rats, DNIC is less efficient in females compared with males, and the brain networks engaged during DNIC differ in males and females.[77] The above discussion of sex differences in pain modulation by vlPAG/DR circuit projections to the BNST is a good example of why it is critical for the nascent alcohol-pain field to include both sexes in all studies.

It is largely unknown how the age of onset for chronic pain affects (1) pain-induced alterations in the central nervous system, (2) neurobiological mediators of the effects of pain on behavior, and (3) the modality, intensity, and duration of chronic pain–related behavior. For example, in the CeA, some studies of chronic inflammatory pain have been performed in adult mice,[52] and others have been performed in adolescent mice.[55] In those studies, CeA cells were sorted and

classified according to firing pattern (e.g., regular spiking, fast spiking, late firing, bursting), and the resulting cell population breakdown differed greatly between the two studies. Thus, even when measuring similar physiological outcomes in vitro in the CeA of mice treated with the same in vivo manipulation (i.e., CFA to induce chronic inflammatory pain), results may vary. There are several possible explanations for these discrepant results, but one potential major contributor to these results is the age of rodents at the time of CFA treatment and sacrifice. In the context of pain-alcohol relationships, age may be especially important because (1) alcohol effects on the central nervous system differ according to age of exposure, and (2) human data show that the relationship between pain severity and alcohol use begins early in life, and that childhood trauma is associated with increased risk of chronic pain in adulthood.[79,80] As mentioned above, chronic alcohol exposure during adolescence produces mechanical hypersensitivity and thermal hyperalgesia that last for many weeks following termination of alcohol exposure, but the underlying neurobiological mediators of these effects are unknown. As more research is devoted to testing the neurobiological mechanisms underlying pain-alcohol interactions, it will be important for the field to pay close attention to sex and age differences.

CONCLUSIONS

Neuroscience has recently turned its attention to understanding the neurobiological mechanisms that underlie pain-alcohol interactions. This new research has primarily focused on the emergence of pain-like states after chronic alcohol exposure using animal models, but it also should focus on alcohol use and alcohol effects in rodents with chronic inflammatory or neuropathic pain. To this point, work has focused largely on circuit and molecular mediators of alcohol-related hyperalgesia. Above, this review discusses examples of recent work in this area, with a focus on reciprocal projections between midbrain and the limbic forebrain (i.e., extended amygdala) as well as some neurochemical mediators (dopamine, melanocortins, and CRF) of pain-related phenotypes after alcohol exposure. This list undoubtedly will grow as more labs begin to work in this area, and it will be important going forward for the field to be mindful of sex and age (as well as species) in study design and data analysis of pain-alcohol interactions.

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Alcohol and Cannabis Use and the Developing Brain

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PURPOSE: Alcohol and cannabis are the most commonly used substances during adolescence and are typically initiated during this sensitive neurodevelopmental period. The aim of this review is to provide a comprehensive overview of the most recent literature focused on understanding how these substances affect the developing brain.
SEARCH METHODS: Articles included in this review were identified by entering 30 search terms focused on substance use, adolescence, and neurodevelopment into MEDLINE, Embase, PsycINFO, ProQuest Central, and Web of Science. Studies were eligible for inclusion if they longitudinally examined the effect of adolescent alcohol and/or cannabis use on structural or functional outcomes in 50 or more participants.
SEARCH RESULTS: More than 700 articles were captured by the search, and 43 longitudinal studies met inclusion criteria, including 18 studies focused on alcohol use, 13 on cannabis use, and 12 on alcohol and cannabis co-use.
DISCUSSION AND CONCLUSIONS: Existing studies suggest heavy alcohol and cannabis use during adolescence are related to small to moderate disruptions in brain structure and function, as well as neurocognitive impairment. The effects of alcohol use include widespread decreases in gray matter volume and cortical thickness across time; slowed white matter growth and poorer integrity; disrupted network efficiency; and poorer impulse and attentional control, learning, memory, visuospatial processing, and psychomotor speed. The severity of some effects is dependent on dose. Heavy to very heavy cannabis use is associated with decreased subcortical volume and increased frontoparietal cortical thickness, disrupted functional development, and decreased executive functioning and IQ compared to non-using controls. Overall, co-use findings suggest more pronounced effects related to alcohol use than to cannabis use. Several limitations exist in the literature. Sample sizes are relatively small and demographically homogenous, with significant heterogeneity in substance use patterns and methodologies across studies. More research is needed to clarify how substance dosing and interactions between substances, as well as sociodemographic and environmental factors, affect outcomes. Larger longitudinal studies, already underway, will help clarify the relationship between brain development and substance use.

KEYWORDS: alcohol; cannabis; adolescence; brain; cognition; neuroimaging

Adolescence is marked by significant social, emotional, cognitive, and physical changes, as individuals transition from childhood to adulthood. Although the exact definition of adolescence tends to vary, recent findings regarding adolescent development and growth include individuals between the ages of 10 and 24.[1] Consistent with this defined age range, the human brain continues to develop until approximately age 25.[2–4] Overall, total brain volume does not change during adolescence; however, there are significant microstructural changes in gray and white matter volume. Specifically, development of gray matter (i.e., neuronal cell bodies, dendrites) follows an inverted U-shaped curve, whereby volume increases until approximately ages 12 to 14, followed by a gray matter decrease due to synaptic pruning, changes in the extracellular matrix, and white matter encroachment.[5–7] In contrast, white matter, which consists of neuronal axon tracts that connect gray matter regions, develops linearly into the mid-20s, as neural connections are optimized.[2,8] Together, these structural changes in gray and white matter between ages 10 and 24 are related to significant socioemotional and cognitive development. Most prominently, emotion and reward-related regions of the brain mature fully during adolescence, while higher-order cognitive functions such as cognitive control, decision-making, planning, and working memory are slower to develop.[2] These neural changes are believed to lead to heightened sensation seeking, impulsivity, and reward responsiveness during adolescence, as well as reduced ability to inhibit emotions and behaviors.[9,10] This imbalance between reward and cognitive control also is believed to contribute to greater risk taking, including the initiation and escalation of substance use.[11] These neural changes leave youth more vulnerable to the potentially serious and long-lasting consequences of substance use.[12,13]

Emerging research supports the notion that substance use disorders are developmental problems that begin during adolescence and have negative consequences on individuals throughout the life span.[14,15] Alcohol and cannabis are the most commonly used substances during adolescence and are typically initiated during this important neurodevelopmental period, with patterns of use ranging from low and infrequent to heavy and problematic.[16] Globally, alcohol is the most commonly used substance with 27% of 15- to 19-year-olds reporting alcohol use in the past month, with rates peaking to 41% for 20- to 24-year-olds.[17] Early alcohol use is related to poorer long-term outcomes; the prevalence of lifetime alcohol use disorder is 41% for those initiating alcohol use by age 12, compared to 17% and 11% for those initiating use at ages 18 and 21, respectively.[18] Cannabis is the second most commonly used substance during adolescence, with overall rates of use increasing globally, particularly in regard to rates of daily use.[19] Past-year cannabis use among 15- to 16-year-olds is highest in the Oceania region (18%), the Americas (12%), and Europe (12%), with rates of use increasing and peaking in 20- to 24-year-olds.[19]

Given the high rates of alcohol and cannabis use during adolescence, coupled with the significant neural maturation occurring during this period, it is critical to understand how alcohol and cannabis use affect adolescent brain development. Although other reviews exist on these topics, they have limitations. Specifically, existing reviews exclusively focus on alcohol,[12,20] cannabis,[21] or co-use,[22] with some focusing solely on neuropsychological[23] or neuroimaging studies[24–27] within each substance use group. The aim of this review is to provide a comprehensive overview of the most recent literature that is both (1) focused on alcohol, cannabis, and alcohol and cannabis co-use use during adolescence and (2) meets the criteria for a prospective longitudinal neuropsychological and neuroimaging study in humans. Limitations of existing studies and future directions for research are discussed.

Search Methods

Articles included in this review were identified via literature searches using MEDLINE, Embase, PsycINFO, ProQuest Central, and Web of Science, conducted on February 19, 2021. To capture the effects of alcohol and/or cannabis use on neural and cognitive development during adolescence, search terms included: (1) alcohol, cannabis, marijuana; (2) adolescen*, teenage*, young people, youth, emerging adult, young adult, college student; and (3) neuroimag*, neuroscience, PET scan, brain imag*, spectroscop*, magnetic resonance imag*, fMRI, sMRI, magnetic resonance spectroscopy, electroencephalogram, diffusion tensor imag*, structural imag*, functiona imag*, neuropsychological test, cogniti*, verbal working memory, episodic memory, visuospatial working memory, verbal fluency test, executive function*. In keeping with previous reviews,[12,28] studies were eligible for inclusion in this narrative review if they met the following criteria: (1) examination of the effect of alcohol and/or cannabis use on neurodevelopment, including brain structure, brain function, and neuropsychological function; (2) longitudinal study with two or more neuroimaging or neuropsychological assessments; (3) adolescent sample ages 10 to 25 at baseline; and (4) sample size of 50 or more participants to reduce the likelihood of spurious findings. Cross-sectional studies are not included.

Results

Overview

More than 700 articles were captured by the search; and 43 longitudinal studies met inclusion criteria, including 18 studies focused on alcohol use, 13 on cannabis use, and 12 on alcohol and cannabis co-use. The effects of alcohol and cannabis use on ongoing adolescent neurodevelopment are described, portioned

by brain structure (i.e., macrostructural and microstructural effects), brain function (i.e., resting state connectivity, task-based neural response), and neuropsychological effects (i.e., executive functions, impulsivity, attention, learning and memory, visual processing, verbal ability, psychomotor speed, IQ). Information on levels and typologies of alcohol and cannabis use (see Figure 1), age, and race/ethnicity details are described where available. To enable comparison across studies, the terms used in each study to describe the level of substance use (i.e., heavy drinking) have been standardized to align with the figure.

Where applicable, sex-specific findings are reported. Studies focused on alcohol effects are summarized first, followed by cannabis, then co-use studies. Two consortium-sized studies have examined the effect of substance use on the developing brain, including IMAGEN[29] and the National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA).[30]

IMAGEN is a multicenter brain imaging study of 2,216 adolescents recruited at age 14 from eight sites in Germany, the United Kingdom, and France. At baseline, 53% of youth reported lifetime alcohol use, 30% had smoked tobacco, and 12% had tried another substance, including 7% who had tried cannabis. NCANDA is a prospective longitudinal study being conducted in the United States across five sites, following 831 youth ages 12 to 21 who were required to have had limited exposure to alcohol at baseline (i.e., ≤ 5 drinking days for youth ages 12 to 15, ≤ 11 drinking days for youth age 16, ≤ 23 drinking days for youth age 17, ≤ 51 drinking days for youth age ≥ 18) or other substances (i.e., ≤ 5 days with cannabis use for youth age 12, with an additional five uses allowed per 1-year increase in age).[30] A number of studies described below utilize subsamples from these data sets.

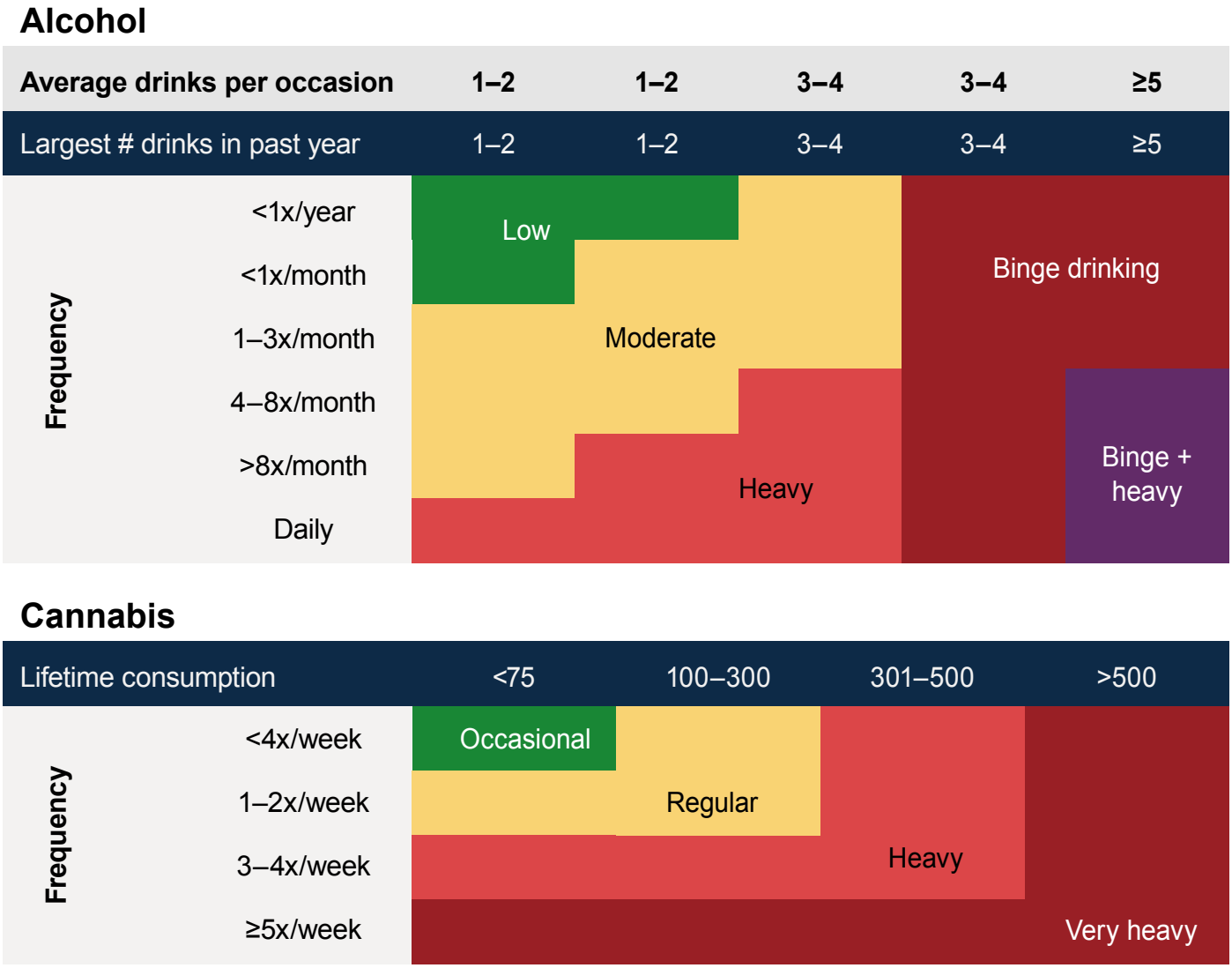

Figure 1. Typology of alcohol and cannabis use during adolescence. The charts are based on existing data classifying substance use groups during adolescence. Cannabis consumption is measured in occasions of cannabis use.[12,28]

Macrostructural Effects on Brain Structure

A number of longitudinal structural magnetic resonance imaging (MRI) studies have explored changes in brain volume and cortical thickness that occur across time following alcohol or cannabis use during adolescence. Several studies have delineated the post-substance use effects on brain structure by comparing youth who have and have not consumed alcohol or cannabis, and some have explored the relationship between levels of use and structural effects.

Alcohol

Among 483 NCANDA participants (baseline mean age = 16; 73% White, 14% Black, 11% Asian, and 2% undisclosed race/ethnicity), a portion of youth initiated moderate (13%) or heavy (13%) drinking by the 2-year follow-up assessment.[31] Youth who drank heavily (see Figure 1) exhibited accelerated decreases in frontal gray matter volume in a dose-dependent manner when compared to controls, who drank little or not at all. Importantly, no significant group differences in frontal brain volume were observed at baseline prior to drinking onset, suggesting that aberrant volumetric trajectories were the result of alcohol uptake. By the 3- to 4-year follow-up assessment (n = 548), 22% of youth were drinking moderately and 18% were drinking heavily.[32] Both moderate and heavy drinkers continued to exhibit altered neurodevelopmental trajectories with a graded dose effect, including accelerated cerebellar gray matter decline, white matter expansion, and cerebrospinal fluid volume expansion relative to controls. Notably, the authors did not explore baseline group differences prior to the onset of alcohol use; thus, pre-existing volumetric differences may be contributing to the observed effects. Interestingly, occasional cannabis co-use did not contribute to the effects in either study.[31,32]

The alcohol effects observed in the NCANDA sample are consistent with three smaller longitudinal studies of adolescents with a mean age of 15 to 18 at baseline (N = 55 to N = 134; 64% to 95% of the samples were White).[33,34] These studies demonstrated that heavy drinking over 2 to 4 years was associated with accelerated decreases in frontal, parietal, and temporal gray matter volume and frontal cortical thickness.[33,34] Additionally, these studies have reported attenuated increases in white matter growth over time for people who drank heavily when compared to the control group, who did not drink.[33,34] One of these studies observed no group differences in cortical thickness or white matter volume at baseline, indicating that the effects were the result of alcohol consumption.[34] However, a follow-up study found that pre-existing differences may partially contribute to neural outcomes among individuals who initiate alcohol use,[35] consistent with previous reviews.[11] Occasional cannabis use (mean days of cannabis use over 3 months before scan = 5) did not contribute to the observed effects in one study.[33]

In contrast to the studies above, findings from the IMAGEN study suggest that drunkenness frequency in 726 participants (100% White) was not associated with gray matter volume between ages 14 and 19 when controlling for sociodemographic, puberty, and substance-related confounding factors.[36] Interestingly, a directionality analysis demonstrated that aberrant development of gray matter in the frontal and temporal regions prior to alcohol use was associated with increased prospective drunkenness frequency throughout adolescence. The discrepancy in findings between studies may relate to the age of participants as well as to differences in pre-existing factors. Robert et al. examined the effects of alcohol use during early adolescence (age 14) when youth had been consuming alcohol for a relatively short period of time,[36] whereas the other studies reviewed here examined the effect during late adolescence (ages 17 to 21) where youth typically exhibited a longer drinking history. Therefore, macrostructural disruption may be a function of greater cumulative alcohol use across adolescence.

Cannabis

A study focused on the effect of adolescent cannabis use in the IMAGEN cohort (n = 706) indicated that greater consumption (i.e., occasional to regular use, see Figure 1) between ages 14 and 19 was associated with reduced expansion of the hippocampus and parahippocampus.[37]

Alcohol and cannabis co-use

Examination of adolescents engaging in heavy and frequent cannabis and alcohol co-use (mean lifetime days of cannabis use = 1,110; mean lifetime days of alcohol use = 605) found that heavy co-use was associated with a global reduction in cortical thinning (i.e., increased thickness) compared to controls, with frontal and parietal lobes being most consistently affected.[38] Given that heavy alcohol use has been associated with decreased cortical thickness, heavy cannabis use may result in a differentiated pattern of macrostructural disruptions throughout late adolescence. Furthermore, one study examined the effect of monitored abstinence from heavy alcohol and cannabis use on macrostructural recovery among youth (N = 54; 76% White) who initiated use during middle adolescence.[39] Participants who engaged in heavy alcohol and cannabis use throughout middle and late adolescence continued to exhibit thicker cortices than controls following 4 weeks of monitored abstinence, consistent with the co-use study described above.[38] Further research is required to determine whether the deleterious effects of substance use on macrostructural development recede following reductions in use.

Microstructural Effects on Brain Structure

Diffusion tensor imaging studies measure the microstructural integrity of white matter by mapping the diffusion pattern of water molecules.[40] Common diffusion tensor imaging metrics

include fractional anisotropy (FA), a measure of diffusion anisotropy or the unidirectionality of diffusion within a voxel; mean diffusivity (MD), a measure of diffusion magnitude; and axial diffusivity, a measure of the magnitude of diffusion parallel to the primary direction of diffusion, which may be a marker of axonal damage. White matter integrity following alcohol and cannabis use was examined in six studies, including four alcohol use studies and two alcohol and cannabis co-use studies.

Alcohol

In a study utilizing 4 years of NCANDA data, a whole-brain FA analysis of 451 adolescents was conducted.[41] Youth who drank heavily (89% White) exhibited greater widespread FA reductions compared to no- and low-drinking controls, with a dose-dependent response observed. Interestingly, alcohol-associated disruptions were greater among youth ages 14 to 19.3 compared to youth ages 19.4 to 25 and were most pronounced in the genu and body of the corpus callosum, regions known to continue to develop throughout adolescence.[42] Here, FA trajectories over 4 years were not correlated with occasional to regular cannabis use. Similarly, a 2-year study of 55 adolescents (95% White) observed relative FA decreases in temporal and subcortical regions among youth who initiated regular alcohol use between ages 17 and 18 relative to non-using controls.[34] Sex differences were observed in one study. In a sample of 113 adolescents who were alcohol-naïve and ages 12 to 16 at baseline, greater alcohol consumption over the 3-year follow-up period was associated with greater FA reductions and mean diffusivity increases in the splenium of the corpus callosum and posterior thalamic radiation among males, and the opposite direction of effects was observed among females.[43] Interestingly, sex hormones partially explained the effect of alcohol use on white matter microstructure.

Alcohol and cannabis co-use

Two studies from the same research group assessed youth who reported alcohol and cannabis co-use, and together they found limited evidence to suggest cannabis is neurotoxic to white matter integrity.[44,45] The first study investigated the effect of continuing heavy cannabis and alcohol use (and occasional other substance use) over 18 months among 92 adolescents ages 16 to 21 at baseline (58% White).[44] Greater alcohol use over the follow-up period was related to higher mean diffusivity bilaterally in the superior longitudinal fasciculus and higher axial diffusivity in the left posterior corona radiata; cannabis use was not correlated with diffusion indices. The second study examined the same cohort over 3 years (*N* = 54, 74% White) and compared older adolescents engaging in concurrent binge drinking and heavy cannabis use to those engaging in binge drinking only or no substance use.[45] Youth in both the binge drinking only and co-use groups exhibited similar widespread FA reductions, with no added deleterious effect observed in the co-use group. Thus, the evidence to date indicates that heavy alcohol use and binge drinking, but not cannabis use, result in neurotoxic microstructural effects among younger and older adolescents.

Summary

Heavy alcohol use during late adolescence is associated with accelerated widespread decreases in gray matter volume and cortical thickness throughout frontal, parietal, temporal, and cerebellar regions. Additionally, attenuated white matter growth and poorer white matter integrity throughout widespread regions have been observed among heavy drinkers, with greater disruptions from consumption during middle to late adolescence than during young adulthood.

Heavy cannabis use may be associated with a differentiated neural pattern than alcohol use alone. Cannabis use is associated with macrostructural consequences only, including reduced expansion of the hippocampal region and increased cortical thickness in the frontal and parietal lobes. Early evidence suggests that disruptions in macrostructural development due to cannabis use do not recede over the short term; however, further research on the recoverability of substance-related macrostructural effects is required. New evidence also highlights the importance of studying sex and sex hormones when investigating the effects of alcohol and cannabis use on the adolescent brain;[43] however, further research is required.

Effects on Brain Function

Longitudinal studies have measured resting-state functional connectivity and neural response to cognitive tasks across time to examine the effect of alcohol and cannabis use on brain function.

Alcohol

Limited evidence is currently available on the specific effects of alcohol use on functional neurodevelopment. A 4-year study examined the effect of low-level alcohol consumption during middle to late adolescence on neural response to cognitive control tasks (*N* = 92).[46] Low-level consumption (< one standard drink [14 grams of alcohol] per week at age 14 to < four drinks per week by age 18) did not impair ongoing maturation of cognitive control networks, with similar increases in activation of the anterior cingulate cortex and pre-supplementary motor area over time among non-drinkers and low-level drinkers. Meanwhile, the effect of heavy alcohol consumption during adolescence was examined in a study utilizing three annual assessments of resting-stage functional MRI data from the NCANDA cohort (*N* = 526).[47] To explicate the specific effects of alcohol use, any clusters correlated with cannabis use were omitted from the analysis. Higher levels of alcohol consumption over the follow-up period were related to greater within-network connectivity in two motor networks, and these effects were mediated by sensation seeking. Interestingly, alcohol use effects were more pronounced in female adolescents than in male adolescents, with a graded dose effect observed.

Cannabis

The effect of prolonged and heavy cannabis use on functional neurodevelopment was examined in four studies. Leveraging the IMAGEN data set, prolonged occasional to regular cannabis use from ages 14 to 19 was associated with relative declines in neural reactivity to angry faces across time when compared to substance-naïve matched controls (n = 76).[48] Of note, this effect was no longer significant when the participants who used cannabis were compared to the larger, unmatched sample of naïve participants (n = 502). In a younger sample of adolescents ages 12 to 15 (N = 67, 67% White), the initiation of occasional cannabis use was associated with decreased activity in the cuneus during visuospatial working memory compared to controls; however, there were no changes to cognitive scores.[49] In a study of 65 youth ages 10 to 23 at baseline (mean age = 17), adolescents engaging in very heavy cannabis use and seeking treatment for cannabis use disorder exhibited a decline in resting functional connectivity between the anterior cingulate cortex and the dorsolateral and orbitofrontal cortices across 18 months.[50] Finally, one study investigated neurofunctional recovery following 4 weeks of monitored abstinence from cannabis after 6 years of very heavy cannabis use (average joints per year = 899; total lifetime days of use = 5,268).[51] Abstinence was associated with a reduction in the magnitude of functional differences between the cannabis use and control groups. Compared to controls, abstinent cannabis users showed a differentiated pattern of connectivity within the insula and default mode networks as well as stronger anticorrelation between them. A graded dose effect was observed, where the extent of persistent alterations in functional activity was related to the amount of cannabis previously used.[51]

Alcohol, cannabis, and tobacco co-use

The effect of any substance use—including alcohol, cannabis, and tobacco—on adolescent brain function was assessed annually across 4 years among 167 adolescents ages 13 to 14 at baseline.[52] Greater substance use over time was related to increased insula activation during risk processing, with more pronounced effects for adolescents with low compared to high cognitive control. This study did not explore independent effects of alcohol and cannabis use on neural activation.

Summary

Overall, preliminary evidence indicates that heavy alcohol use during adolescence disrupts the maturation of network efficiency in a dose-dependent manner, with more significant effects observed among females. Even relatively low-level cannabis use (i.e., occasional and regular consumption) as well as heavier use during adolescence may alter the rate of neurotypical functional development in brain regions important for cognitive control. Some neural recovery may be possible after abstinence; however, months or years may be required for complete recovery of functional connectivity from heavy cannabis use. Preliminary evidence underscores that cognitive control and sensation-seeking behaviors could be an important target in prevention and treatment of substance use in adolescents, given the moderating roles on neurofunctional effects. Further research is required to determine the relative effects of alcohol and cannabis consumption on functional neurodevelopment and whether neural recovery occurs following reductions in use.

Effects on Neuropsychological Function

Neuropsychological tests enable tracking of cognitive skills over time to uncover the effect of alcohol and cannabis use on cognitive development. The following sections summarize the reported effects by neuropsychological domain.

Executive functions

Executive functions refer to a range of top-down mental processes that enable an individual to hold concentration and attention. There are three core executive functions, including inhibition, working memory, and cognitive flexibility.[53,54] From these core functions, other higher-order executive functions are built, such as reasoning, problem-solving, decision-making, and planning.[55] Mediated by frontal lobe development, these functions are essential for educational and occupational success, mental and physical health, and social development.[55] Current evidence suggests that alcohol consumption during adolescence does not impair maturation of executive functions. A 4-year study of 92 adolescents found that greater cumulative low-level alcohol consumption (< four drinks per week at age 18) between ages 14 and 18 did not have an effect on conflict monitoring or updating of working memory performance and was associated with subtle improvements in inhibitory control.[46] Likewise, a study using data from 2,226 adolescents in the Tracking Adolescents' Individual Lives Survey (TRAILS) found that 4 years of occasional or frequent low or heavy alcohol use was not associated with deterioration in inhibition, working memory, or cognitive flexibility, compared to no alcohol use.[56,57] In this study, cannabis use at ages 16 and 19 was not correlated with executive functioning performance across ages 11 to 19. Lastly, a 4-year study of 234 adolescents unexpectedly found that more alcohol use predicted better working memory, driven largely by a positive relationship between recent blackout history and auditory attention scores, when controlling for sociodemographic factors.[58] Notably, no follow-up tests supported the unexpected working memory finding, such as removing sex and other covariates from the regression models.

Similarly, most studies examining young adults have not found detrimental effects of alcohol use on executive functioning development. A 4-year study followed 155 young adults every 22 months from age 18. Individuals who reported consistent binge drinking throughout the entire study showed no disadvantage for decision-making ability when compared to non–binge-drinking controls, who consumed four drinks per week

on average.[57] Here, occasional cannabis use was not related to decision-making ability. Meanwhile, an assessment of 436 Dutch young adults (mean age = 21 years) showed baseline alcohol use of any level (i.e., abstinence, occasional moderate, frequent moderate, occasional heavy, frequent heavy) was not related to planning or reasoning ability 11 months later, nor was change in average alcohol consumption over time.[59] Finally, another study assessed 89 young adults ages 18 to 20.[60] Compared to non–binge-drinking controls, individuals who reported consistent binge drinking over 2 years exhibited poorer conflict monitoring at both time points. However, consistent binge drinking over 2 years was not associated with deterioration in working memory or planning across time. Additionally, occasional cannabis use was not associated with performance.

As described above, studies that focused on the impact of alcohol use have not reported an effect of occasional cannabis use on executive functioning maturation throughout adolescence and young adulthood.[56,57,60] Meanwhile, the Co-Venture study assessed 3,826 adolescents with a mean age of 13 at baseline who were assessed annually for 5 years.[61] Cannabis use ranged in frequency from occasional to very heavy use (i.e., daily); and when accounting for alcohol use, the female cannabis users were shown to be more sensitive to negative consequences of working memory than were the males. Data from the Dunedin Study of 1,037 individuals showed that adolescent-onset and persistent very heavy cannabis use was associated with impaired working memory and perceptual reasoning over more than 20 years.[62] Another study assessed 175 adolescents ages 12 to 15 at baseline across the course of 14 years.[63] Greater cumulative cannabis use over adolescence was associated with poorer inhibitory control. Finally, a study assessing the effect of cannabis use among 58 young adults age 19 over a 2-year period (82% White) found that cannabis consumption declined from very heavy to heavy, which corresponded to improvements in working memory, planning, and motivated decision-making, suggesting that deficits may be associated with very heavy use only and that these higher-order cognitive functions are recoverable following reductions in consumption.[64]

Overall, there is no strong, consistent evidence to indicate that low to heavy alcohol use during adolescence or young adulthood disrupts executive functioning maturation across time. Longitudinal data on cannabis use and executive functioning performance suggest that frequent consumption and greater cumulative use across adolescence may disrupt inhibitory control, working memory (particularly in females), planning, and decision making.

Impulsivity

Impulsivity is defined as a behavior characterized by little or no forethought, reflection, or consideration of consequences, when compared to actions by individuals with similar skill and knowledge levels. Impulsivity is thought to be related to risk-taking behaviors.

Two studies examined the impact of alcohol use on impulse control across adolescence; however, no studies have examined the impact of cannabis use or co-use of these substances. IMAGEN data from 304 young people ages 13 to 14 at baseline found that over a 2-year period, adolescents who reported more than 40 occasions of alcohol use exhibited increases in trait impulsivity, while youth who reported alcohol use on fewer than 10 occasions exhibited decreases in impulsivity.[65] Likewise, a study of 116 adolescents with an average age of 14 at baseline demonstrated that greater total lifetime drinks over approximately 2 years predict escalated impulsive choice across time.[66] In both studies, limited cannabis use was reported. Therefore, the transition into frequent drinking in early to middle adolescence may disrupt normative developments in impulse control.

Attention

Attentional control has been measured in two longitudinal studies focused on the effects of low to heavy alcohol use; in two studies focused on effects of heavy cannabis use; and in three studies exploring co-use of alcohol and cannabis.

The TRAILS study of 2,226 adolescents reported that 4 years of weekly low or heavy alcohol use did not have an effect on sustained attention, when compared to controls who consumed no alcohol.[56] However, sex differences were identified in a 5-year study of 89 adolescents age 14 at baseline (76% White), where more hangover symptoms (from heavy alcohol use) in the previous year predicted relative worsening of sustained attention in males only.[67] Heavy cannabis use did not predict change in attention across time in this study.

In terms of cannabis-related effects, declines in cannabis use from very heavy to heavy consumption correspond with improvements in attention.[64] Likewise, a study of 74 youth ages 16 to 26 (66% White) found that 2 weeks of monitored abstinence from very heavy cannabis use was associated with improvement in attention compared to controls.[68] Together, these data suggest that very heavy cannabis use during adolescence and young adulthood is associated with diminished attention; however, such deficits may recover following reductions in use.

Additionally, alcohol and cannabis co-use has been associated with progressive declines in attentional control across time. In a study of 69 adolescents (80% White) observed from ages 13 to 19, the initiation of concurrent use was related to deficits in complex attention compared to substance-naïve counterparts.[35] A negative dose-response relationship also has been observed over an 8-year period from ages 16 to 24 (78% White), where greater co-use of cannabis and alcohol among 73 adolescents was related to poorer attention.[69] Interestingly, when assessing the relative effects of concurrent heavy alcohol and cannabis use over 3 years among 108 adolescents (63% White), attentional differences appeared to be driven by alcohol rather than cannabis use.[38]

In summary, previous studies have identified attentional deficits among heavy drinking males and heavy cannabis users. Initiation of co-use of these substances in adolescence has predicted poorer attention, with graded dose effects observed that may be driven by alcohol use. Early evidence suggests that adolescents may recover from cannabis-related effects following reductions in use. Recoverability from alcohol effects remains unknown.

Learning and memory

Inextricably linked to adolescent learning and memory development is educational attainment, one of the most critical developmental tasks for youth. Thus, substance-induced deficits are arguably even more impactful for young people than adults. Ten studies included in this review examined the effect of alcohol or cannabis use on learning and memory performance throughout adolescence.

Alcohol-focused studies have predominantly reported on the impact of heavy binge drinking. A 6-year study of 112 substance-naïve adolescents (mean baseline age = 13; 69% White) found that higher estimated peak blood alcohol concentration over the 3-month period before the follow-up neuroimaging session predicted worse verbal learning and immediate, short- and long-term delayed, and cued recall across time in a dose-dependent manner.[70] Furthermore, a 6-year study following 155 older adolescents every 22 months from age 18 found that consistent binge drinking was associated with deficits in immediate and delayed recall, with similar deficits for males and females when compared to non–binge-drinking controls.[71] Occasional cannabis use did not influence the effects. Similarly, previously described studies assessing the impact of the frequency of drinking days in middle adolescence[58] and consistent binge drinking in late adolescence[60] have observed poorer performance on immediate and delayed recall as well as on retention after 2 to 4 years of continued use. In contrast to these findings, one study reported that occasional or frequent alcohol use at moderate or heavy levels was not related to short-term delayed recall performance 11 months later among young adults with a mean age of 21.[59] However, study authors note that the null findings should be interpreted with caution given the high variance in cognitive performance. Two studies have focused on the effect of adolescent cannabis use on learning and memory performance. One study examined the impact of early (< age 16) and late (≥ age 16) onset of cannabis use on learning ability among 119 young people (89% Black).[72] On one of four tests, early-onset cannabis use was associated with a small decline in structured learning performance compared to no use; however, neither group exhibited suboptimal learning trajectories on the majority of tests. Additionally, in a large representative cohort of young adults ages 20 to 24 at baseline (n = 1,978), occasional cannabis use was associated with decreased immediate recall compared to young people with long-term abstinence from cannabis use, suggesting recovery may be possible after long-term abstinence.[73]

Alcohol and cannabis co-use has been shown to impair learning and memory, with preliminary evidence implicating alcohol as the predominant driver of these effects. The effect of heavy alcohol and cannabis use (where participants met criteria for alcohol use disorder and engaged in other substance use) on learning and memory trajectories across 10 years was examined during middle to late adolescence.[74] Examining 213 participants, heavier use patterns and greater hangover and withdrawal symptoms over time were related to poorer verbal learning and memory, suggesting a dose-dependent relationship between substance use and cognitive functioning. Similarly, a second study showed that adolescents with a history of substance use disorder (concurrent alcohol, cannabis, and stimulant use) demonstrated impairments in verbal learning and memory compared to youth without substance use disorder, when followed up seven times from ages 16 to 24 (N = 73, 78% White).[69] Finally, a previously described study showed that adolescent engagement in concurrent heavy cannabis use and binge drinking over 18 months was associated with progressive declines in delayed recall when compared to those engaging in occasional cannabis use alone.[38] Further analysis of this cohort at the 3-year follow-up where groups reported congruent levels of alcohol use suggested that the memory deficits may be a result of alcohol rather than cannabis use.

Overall, studies focused on alcohol use during adolescence have observed a disruption in learning and memory development following heavy and binge drinking, with the severity of effects related to levels of consumption. Occasional cannabis use has been shown to have a negative effect on recall but not on learning. Meanwhile, heavy co-use for up to 10 years is related to poor outcomes, which may be driven by the effects of alcohol use rather than cannabis use.

Visual processing

Visual processing involves the brain's analysis and interpretation of visual signals. Seven previously described studies have examined the impact of alcohol and cannabis use on visual processing ability across adolescence, including four alcohol-focused studies and three co-use studies.

Initial evidence from the previously described study suggests that low-level alcohol use during adolescence does not have a negative effect on the development of rapid visual processing.[46] In contrast, heavy alcohol use and withdrawal symptoms during middle to late adolescence have been associated with prospective declines in visuospatial function over 10 years, compared to controls.[75] Additionally, a dose-dependent effect has been observed among 234 adolescents ages 12 to 14 at baseline, where greater number of drinking days over 4 years predicted visuospatial ability.[58] Examination of sex differences suggests that this effect may be particularly strong among young females.[67]

Others studies have found that adolescent engagement in heavy cannabis use and binge drinking over 3 years has

resulted in significant declines in visuospatial functioning, with effects driven by alcohol use.[38] Moreover, greater cumulative cannabis use over 14 years and proximal increases in alcohol consumption predict decrements in visuospatial functioning.[63] Notably, 4 weeks of monitored abstinence from concurrent cannabis use and binge drinking were not associated with improvements in visuospatial functioning.[39] Overall, there is consistent evidence that heavy alcohol use during middle to late adolescence leads to poorer visual processing and functioning. Performance does not appear to improve over the short term following a period of abstinence.

Verbal ability

Verbal ability refers to the ability to both understand and communicate effectively with words. Comprehension and verbal fluency are considered parts of verbal ability.

Two large cohorts of twins (cohort 1, *n* = 2,277; cohort 2, *n* = 1,241) show that the initiation of occasional cannabis use was associated with a decline in verbal ability; however, this finding is not apparent in twins discordant for cannabis use (cohort 1, *n* = 94; cohort 2, *n* = 200).[76] Additionally, persistent very heavy cannabis use over 20 years was predictive of impaired verbal comprehension (*n* = 1,037).[62] No studies included in this review examined the effect of alcohol use or alcohol and cannabis co-use on verbal ability across adolescence.

Psychomotor speed

Psychomotor speed is defined as the relationship between cognitive and motor movements, often measured by both accuracy and speed. It includes movement, spatial relationships, and use of motor skills.

Preliminary evidence shows that alcohol and cannabis use in middle adolescence affects psychomotor development. Among 234 adolescents ages 12 to 14 at baseline, several substance use behaviors predicted psychomotor speed performance 4 years later.[58] Specifically, more post-drinking effects from heavy-level alcohol use and greater substance use (including cannabis) was associated with slower psychomotor speed.

IQ

IQ is a standard measure of an individual's intelligence level. Four studies included in this review examined the effect of occasional to very heavy cannabis use on IQ across adolescence. Two large cohorts of twins showed that the initiation of occasional cannabis use was associated with a decline in IQ; however, this finding was not apparent in twins discordant for cannabis use,[76] suggesting IQ deficits may be attributable to confounding factors rather than the direct neurotoxic effect of cannabis. Similarly, another large study of twins (*N* = 1,989) demonstrated that the initiation of regular cannabis use was not associated with prospective IQ decline in discordant twins for cannabis use.[77] The effect of heavier cannabis exposure on IQ was examined in a third study. When comparing 65 adolescents ages 17 to 20 who were current very heavy users (≥ 5 joints per week), current heavy users (< 5 joints per week), former users (no regular use for ≥ 3 months), and non-users, only the group with very heavy cannabis use showed any relative IQ decline across 8 years.[78] Likewise, an additional study reported that adolescent-onset, persistent very heavy cannabis use over 20 years was associated with IQ declines across time (*N* = 1,037).[62] No studies included in this review examined the effect of alcohol and cannabis co-use on IQ across adolescence.

Summary

A wealth of longitudinal studies have assessed the effect of adolescent alcohol and cannabis use on neuropsychological development. Based on the current evidence base, heavy alcohol use (including binge drinking) during adolescence disrupts normative developments in impulse and attentional control, learning and memory, visual processing and functioning, and psychomotor speed, with the severity of some effects dependent on dose. In contrast, low to heavy alcohol use during adolescence and young adulthood does not appear to disrupt executive functioning maturation across time. The recoverability of alcohol effects generally remains unknown.

Longitudinal data on cannabis use and neuropsychological development are generally lacking. Preliminary evidence suggests that heavy to very heavy use could lead to deteriorated development of executive functions and IQ. Heavy alcohol and cannabis co-use in adolescence has been linked to a range of deficits, including deficits in attentional control, learning and memory, visuospatial functioning, and psychomotor speed. The added effect of co-use versus singular use has not been adequately explored to date, although early evidence suggests that heavy alcohol use may be driving some of these effects.

Discussion

The rapidly expanding literature of prospective, longitudinal studies tracking neurodevelopment and substance use has greatly increased knowledge of the effects of adolescent alcohol and cannabis use on brain structure, function, and cognition. Overall, it is clear that heavy alcohol use during adolescence is associated with neural and cognitive consequences (see Table 1). Although there is evidence to suggest that heavy cannabis use can affect ongoing neurodevelopment, early data from co-use studies indicate that alcohol could be partially driving these effects. Parsing out the interactive effects of alcohol, cannabis, and other substances is a key challenge in this field given that other substance use is often accompanied by alcohol use. Basic science and the large multisite human studies currently underway (i.e., IMAGEN, NCANDA, Adolescent Brain Cognitive Development [ABCD] Study) will help disentangle the neural and cognitive effects over the next decade. It is critical to differentiate substance-specific effects, especially given the growing legalization of cannabis use, the upsurge in adolescent

vaping, and global concerns regarding opioid misuse.[79,80] Further, understanding the recoverability from these effects following reductions in substance use is particularly important given the critical focus on continued educational attainment, learning, and ongoing neurodevelopment during adolescence.

An important observation from the current review is the need for more diverse samples. The vast majority of existing work has studied White youth from high socioeconomic backgrounds in the United States and Europe, limiting the generalizability of findings. Future studies also should improve racial descriptions of participants. Often studies report on the proportion of White versus non-White youth, with critical details of race and ethnicity representation overlooked. Another consideration likely reducing the generalizability of the current evidence base is the frequently reported eligibility criteria that excludes youth with co-occurring psychological and medical issues. Importantly, this has enabled specific examination of the effect of substance use on neurodevelopment; however, future studies should begin to explore the interactive effects of adolescent substance use and psychopathology on adolescent neurodevelopment. This knowledge will benefit practitioners working with adolescents and inform future initiatives on substance use prevention and mental health.

Overwhelmingly, the majority of studies thus far have examined effects related to low-level substance use initiation or heavy, frequent use. Although some studies report dose-dependent effects, greater clarification is needed to determine whether there is a threshold for harmful use that results

Table 1 Effects of Adolescent Alcohol and Cannabis Use on the Developing Brain

Size of Effect	Heavy Alcohol Use/Binge Drinking	Heavy Cannabis Use	Alcohol and Cannabis Co-Use
Brain structure			
Small to moderate	• Disruptions observed in middle to late adolescence • Widespread decreases in gray matter volume and cortical thickness • Slowed white matter growth • Poor white matter integrity, partially explained by differences in sex hormones	• Decreases in subcortical volume • Increases in frontoparietal cortical thickness • Neurodevelopmental disruptions may not recover over the short term	
Small to large			• No added deleterious effect of co-use on white matter integrity vs. alcohol use only
Brain function			
Small			• Altered neural response in the insula during risk processing
Small to moderate	• Disrupted maturation of network efficiency • More significant effects among females		
Small to large		• Altered rate of functional development in brain regions important for cognitive control • Some neural recovery possible after abstinence	
Neuropsychological function			
Small to large	• Disruptions in development of: ▪ Impulse and attentional control ▪ Learning and memory ▪ Visual processing and functioning, particularly in females ▪ Psychomotor speed	• Disrupted executive functioning development, particularly in females • Decreased IQ with very heavy use • Improvements in working memory, planning, decision-making, and attention following reduced use	• Attention deficits • Poor psychomotor speed • Progressive declines in learning, memory, and visuospatial functioning (driven by alcohol use) • Short-term abstinence not associated with improved visuospatial functioning

in neural and cognitive consequences. The magnitude of neurodevelopmental consequences from alcohol and cannabis use is likely to stem from a multitude of other factors including sociodemographic characteristics, early-onset puberty, genetic polymorphisms, prenatal exposures, childhood adversity, and psychopathology, among other important factors, which may be lost in the standard mean group values used in analysis.[81,82] Improved quantification of individual variation, as well as exploration of possible interactive effects and underlying mechanisms of neurodevelopmental consequences, are necessary to advance identification of youth who may be at risk for long-term negative effects.

Given ethical barriers surrounding adolescent substance use, this field of research is reliant on observational human studies, which creates challenges for establishing causality and directionality. This review aims to identify neurobiological and neuropsychological consequences of adolescent alcohol and cannabis use by summarizing prospective, longitudinal studies that repeatedly assess individuals over time as patterns of substance use emerge and escalate. However, many of the included studies used only two neuroimaging or neurocognitive time points, which does not allow for more complex modeling and understanding of developmental trajectories over time. Furthermore, reliably identifying causal mechanisms in observational studies without randomization is difficult, with the primary concern being confounding (i.e., whether causal associations are real, or entirely or partly confounded by other variables). The studies synthesized in this review included statistical models with a range of sociodemographic and environmental covariates to address the issue of confounding. However, numerous methods are now available in response to the confounding problem in observational data, such as Granger causal models, structural equation models, Bayesian networks, state-space models, regression discontinuity design, the difference-in-differences approach, and instrumental variable approaches.[83] These techniques have the ability to improve causal understanding and should be utilized in future analyses of large-scale cohorts to delineate causal effects of alcohol and cannabis use.

An additional methodological concern identified in this review is the reliance on youth self-report of substance use. Several studies also used ranges in surveys to capture frequency and quantity of consumption, weakening the ability to explore graded dose effects. Utilization of real-time measures and biological markers can greatly increase the accuracy and reliably of substance use data.[84,85] Although the reported studies focused on alcohol and cannabis use, polysubstance use (e.g., tobacco, cocaine, opioids) could affect findings. Although some studies controlled (or excluded participants) for co-occurring use of other substances, future studies with larger samples will be able to better understand the potential compounding effects of other substance use on brain development. Much of the data presented was collected before vaping existed; given the recent uptick in tobacco vaping, it will be important that future studies assess tobacco vaping to understand its unique effects on adolescent brain development. Furthermore, a greater selection of neuroimaging tools that track neurochemicals and transmitters in the brain (e.g., magnetic spectroscopy imaging, positron emission tomography) are now available. Understanding neurochemical changes could further improve understanding of the mechanisms underlying neural effects of substance use.

Cannabis potency has increased substantially over the past several decades.[86] Quantifying cannabis use is a complex issue due to the lack of regulation and standardization in cannabis products.[87] Most existing studies utilize crude measures of cannabis use (e.g., range of self-reported days of use over restricted periods of time), limiting the ability to understand dose-, time-, and potency-related relationships between cannabis use and neurodevelopmental outcomes. Notably, the National Institutes of Health has recently established a standard 5 mg delta-9-tetrahydrocannabinol (THC) unit to be used in research.[88] Future studies should utilize this unit measurement and incorporate a more granular level of self-report data, as well as objective biomarkers of cannabis use, in an attempt to better understand how potency and quantity of use affects neurodevelopmental outcomes.[89]

Conclusions

In summary, alcohol and cannabis are two of the most commonly used substances during adolescence, which is a critical developmental period associated with significant neurocognitive maturation. Longitudinal neuroimaging and neuropsychological research have helped clarify the effect of substance use on adolescent brain development. Existing studies suggest alcohol and cannabis use during adolescence are related to small to moderate disruptions in brain structure and function, as well as neurocognitive impairment (see Table 1). Overall, findings suggest more pronounced effects related to alcohol versus cannabis use; however, several limitations exist in the literature. Sample sizes are relatively small and demographically homogenous, with significant heterogeneity in substance use patterns and methodologies across studies. More research is needed to clarify how substance dosing and interactions between substances, as well as sociodemographic and environmental factors, affect outcomes. Larger longitudinal studies, already underway, will help clarify the relationship between brain development and substance use. Findings can be used to inform psychoeducational programming[90,91] and provide important targets to developing substance use treatments for adolescents.[92]

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Cognitive-Affective Transdiagnostic Factors Associated With Vulnerability to Alcohol and Prescription Opioid Use in the Context of Pain

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The use of alcohol and prescription opioids is common among people in pain and poses significant public health burdens. This review identifies factors associated with motivation to use alcohol and prescription opioids in the context of pain. Pain-relevant, cognitive-affective, transdiagnostic vulnerability factors—expectancies/motives, pain catastrophizing, pain-related anxiety, distress intolerance, anxiety sensitivity, and perceived interrelations—were selected from theoretical conceptualizations of pain and substance use. Searches conducted in PubMed, PsycINFO, and Embase returned 25 studies that examined associations between identified variables of interest and the use of alcohol and prescription opioids in the context of pain. Consistent with a larger literature on pain and substance use, the studies included in this review demonstrated that people with chronic pain are motivated to use alcohol and opioids in response to negative affect and hold expectancies/motives for coping with pain. Vulnerabilities that engender difficulty managing aversive internal states (distress intolerance and anxiety sensitivity) and maladaptive responses to pain (pain-related anxiety and pain catastrophizing) also were implicated in motivation for alcohol and opioid use. Although one study found that pain-related anxiety was associated with co-use of alcohol and opioids, no studies examined simultaneous use. Future research directions that can explicate causal associations, identify patterns of alcohol and opioid co-use, clarify the role of pain in cessation processes, and inform treatment development are discussed.

KEYWORDS: alcohol drinking; analgesics; opioids; pain; motivation; alcohol

Pain is a complex, near-universal phenomenon, which can be conceptualized as a motivational state that engenders goal-directed action.[1] Motivational models of substance use highlight the role of expected effects and suggest that individuals become motivated to use substances when such use is perceived as holding greater value than other available objects or events.[2,3] A rapidly growing empirical literature indicates that the use of substances, including alcohol and prescription opioids, may be a risk factor in the onset and progression of painful conditions, and that pain is a proximal determinant of acute substance administration and may serve as a barrier to cessation.[4–6] Accordingly, an evolving reciprocal model suggests that associations between pain and substance use are likely bidirectional in nature, resulting in the maintenance and worsening of both conditions over time.[4–6] A recent critical review highlighted emerging evidence that chronic pain frequently co-occurs with use of alcohol and opioids, and that co-use (i.e., use of both substances within a given timeframe) likely contributes to opioid overdose-related morbidity and mortality and worse substance-related treatment outcomes.[7] An important next step in this line of research is to identify potentially modifiable cognitive-affective factors that may underlie or exacerbate motivation to use alcohol and prescription opioids in the context of pain. A focus on processes that contribute to the onset, maintenance, or exacerbation of multiple psychiatric disorders (i.e., "transdiagnostic" factors) can further inform novel treatment targets and intervention development.[8,9]

The sections that follow begin with a brief overview of alcohol and opioid use, acute and chronic pain, and guiding theoretical frameworks. The results of studies that examined associations between pain, selected transdiagnostic cognitive-affective factors (derived from prominent theoretical conceptualizations of pain–substance use relations), and alcohol/prescription opioid use patterns/trajectories are then reviewed. Finally, the relevant extant literature is discussed with an emphasis on explicating clinical implications and generating recommendations to help guide future research in this emerging domain.

ALCOHOL AND PRESCRIPTION OPIOID USE

Prevalence and Impact

Approximately 50% of American adults consume alcohol each month,[10] and more than 25% endorse hazardous drinking (i.e., patterns of use associated with increased risk for harmful consequences).[11,12] Alcohol is implicated in nearly 100,000 deaths in the United States each year,[13] is the third leading cause of preventable death,[14] and has an annual economic impact of more than $250 billion in lost productivity, health care costs, and criminal justice expenses.[15] Although opioid prescribing has diminished somewhat in the wake of the opioid epidemic, nearly 20% of all Americans received an opioid prescription in 2017.[16] Nationally representative data further indicate that more than 12 million Americans misuse prescription opioids each year (i.e., use without a prescription or for a reason other than the purpose for which they were prescribed).[17] In the United States, prescription opioids are responsible for more than 15,000 overdose deaths[18] and for an economic burden of greater than $78 billion annually.[19] Although alcohol and prescription opioids have different pharmacokinetic profiles and substance-specific physiological/subjective effects, they may engender overlapping effects in the central and peripheral nervous systems, including activation of neural circuitry involved in pleasure and reward.[20] Both substances also are implicated in substance use disorders, which are characterized by maladaptive physiological (i.e., tolerance and withdrawal) and behavioral (e.g., impaired control over use behavior, social impairment as a result of use) consequences of use.[21]

Alcohol and Opioid Co-Use

Although definitions vary in the literature, in the context of alcohol and opioids, co-use may be characterized as concurrent use (i.e., within a given period of time, such as past month or past year) or simultaneous use (i.e., co-ingestion at the same time or in a closely overlapping period of time).[22–24] Despite contraindications for drinking alcohol while using prescription opioids, emerging data suggest that the prevalence of alcohol and opioid co-use is surprisingly high. For example, in samples recruited from primary care clinics, 36% of patients with a prescription for daily use of an opioid reported consuming alcohol in the last 30 days,[25] and 9% reported drinking to intoxication on up to 5 days in the past month.[26] Another study of patients on long-term opioid therapy found that 12% reported drinking alcohol within 2 hours of taking their medication.[27] Results from a community-derived sample further indicated that individuals who endorse prescription opioid misuse also report using alcohol at high rates, with up to 20% admitting to using alcohol and opioids in the same day.[28] Finally, nationally representative data indicate that individuals in the United States who meet diagnostic criteria for opioid use disorder (OUD) are nearly twice as likely to also meet criteria for alcohol use disorder (AUD), relative to individuals without OUD.[29] Both alcohol and opioids are central nervous system depressants, and any form of co-use could lead to dangerous, potentially fatal effects (e.g., liver damage, respiratory depression).[30] Indeed, co-use of alcohol was involved in 15% of deaths attributed to prescription opioid overdose in 2017.[31] In addition to heightened morbidity and mortality, a recent critical review found that co-occurring OUD and AUD were associated with poorer treatment outcomes for both disorders, with some evidence that alcohol consumption may increase during medication-assisted treatment for OUD.[7]

Prevalence and Impact of Pain

Although a handful of documented cases indicate a rare congenital inability to perceive pain,[32] pain is largely thought of as a universal human experience.[33] Pain is a highly prevalent public health burden that motivates 50% of annual physician visits in the United States,[34] with chronic pain engendering an annual economic impact of more than $600 billion in health care costs and lost productivity.[35] A recent update by the International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."[36] This definition acknowledges that pain is a complex biopsychosocial phenomenon that involves an interplay of sensory-physiological, cognitive-affective, and behavioral processes, and that the experience of pain cannot be reduced to the activity of sensory neurons. As such, pain may persist beyond expected healing time or in the absence of identified tissue damage. The term chronic pain is typically used to describe pain lasting for at least 3 to 6 months,[37] and is distinguished from cancer-related pain, which differs in etiology and course.[38] More than 100 million Americans have a chronic noncancer pain diagnosis,[39] and recent nationally representative data indicate that on most days, nearly 20 million U.S. adults experience pain that interferes with activities of daily living.[40] The experience of pain commands attention and motivates action to avoid or limit bodily harm, often resulting in avoidance behaviors that can be adaptive in the short term (e.g., to promote healing).[1] However, long-term cycles of maladaptive cognitive-affective responses that lead to persistent avoidance (e.g., worry that pain will never end) are thought to be a predominant cause of pain-related disability.[41] Indeed, chronic pain can have significant negative effects on quality of life and emotional well-being, including interference in occupational functioning,

recreational activities, relationships, self-care, physical activities, and sleep.[42]

Co-Occurring Pain and Use of Alcohol and Opioids

The extant literature suggests that pain co-occurs at high rates with both alcohol and opioid use, potentially in a dose-dependent fashion.[6] With regard to alcohol use, individuals with chronic pain endorse higher rates of hazardous drinking and are up to two times more likely than the general population to meet criteria for AUD.[6,39,43] Greater levels of pain severity[44] and functional interference have been associated with an increased likelihood of engaging in hazardous drinking patterns and meeting diagnostic criteria for AUD,[45] respectively. Pain also appears to be more common among individuals who report hazardous alcohol use: 43% of people who experience drinking problems (e.g., adverse consequences or life problems as a result of drinking) and 75% of individuals with AUD have been shown to endorse current moderate to severe pain (vs. 18% in the general population).[46–48] The co-occurrence of pain and prescription opioid use is intuitive given that opioids are prescribed for pain relief, though pain is also common among individuals who report use of opioid analgesics without a prescription.[49–51] A review of pain and prescription opioid misuse found that 49% to 96% of patients seeking treatment for prescription opioid misuse reported chronic pain, and that 82% reported pain as their reason for initiating opioid misuse.[52] This review further observed a positive association between pain severity and opioid misuse, such that even among patients with chronic pain, pain ratings tended to be higher among those who reported prescription opioid misuse or who met criteria for OUD.

GUIDING THEORETICAL FRAMEWORKS

Pain Processing and Negative Reinforcement

Pain is an inherently aversive experience that reliably elicits negative affect and motivates escape/avoidance behaviors.[1,41] Consistent with biopsychosocial conceptualizations of pain, the four-stage model of pain processing posits that negative affect mediates behavioral responses to pain.[4,53] More specifically, this model invokes both acute negative affect (e.g., distress) that is elicited from the immediate sensory experience as well as extended pain affect (e.g., depression, anxiety) that manifests in the context of chronic pain and functional impairment. Both forms of pain-related negative affect are considered sufficient to motivate adaptive and/or maladaptive behavioral efforts to cope with pain; substance use has been identified as a commonly employed maladaptive pain coping response;[4,54] and efforts to modulate affect have been implicated in pain and substance use trajectories.[41,55] Negative reinforcement—the process by which behavioral responses that are perceived to alleviate aversive states become more likely or increase in frequency—is a core component of theoretical models of both pain and substance use.[1,55] Researchers have further hypothesized that as attempts to avoid/reduce pain or negative affect via substance use are reinforced, the use of substances as a primary coping strategy may become more entrenched over time.[5]

Reciprocal Model of Pain and Substance Use

In drawing upon motivation theory, negative reinforcement frameworks, and the four-stage model of pain processing, a leading reciprocal model posits that pain and substance use interrelate in the manner of a positive feedback loop, resulting in more severe pain, greater functional impairment, and the maintenance of addiction.[4,5] Within this model, the substance use–to–pain pathway acknowledges that although substances such as alcohol and opioids can reduce

pain in the short term, chronic substance use has been identified as a unique risk factor in the onset and progression of hyperalgesia (i.e., increased sensitivity to painful stimuli) and persistently painful conditions.[56–60] In the pain–to–substance use pathway, which is most germane to the current review, pain is conceptualized as a potent motivator of substance use. Pain severity has consistently been associated with use of multiple substances (e.g., nicotine/tobacco, cannabis, alcohol),[5] and human experimental research has shown that pain and pain-related negative affect can increase craving and motivate substance use.[61–63] Indeed, the role of pain as a proximal antecedent to substance use is of growing empirical interest, as highlighted by a recently published Catastrophizing, Anxiety, Negative Urgency, and Expectancy (CANUE) model that emphasizes the influence of negative affect in motivation to self-medicate one's pain with a variety of addictive substances.[64] Thus, the reciprocal model of pain and substance use predicts that acute pain serves as a proximal determinant of substance use behavior, and that via repeated exposures and reinforcement, relations between pain and substance use may become more robust in the context of chronic or persistent pain.[5]

Use of Alcohol and Prescription Opioids in the Context of Pain

Given the high degree of co-occurrence and significant individual/societal costs associated with alcohol and prescription opioid use, the goal of this review is to explicate potentially modifiable factors that are associated with motivation to use and co-use alcohol and prescription opioids in the context of pain.[4,5,64] The need to examine pain as a determinant of alcohol and prescription opioid use is further supported by evidence that nearly 25% of patients enrolled in both pain and inpatient substance use treatment programs have endorsed using alcohol to cope with pain, with many citing pain as the primary impetus for hazardous drinking and other substance use.[65,66] Similarly, a systematic review of opioid misuse and chronic pain found that approximately 21% to 29% of patients prescribed opioids for chronic pain engage in misuse (i.e., use other than prescribed).[67] The CANUE model further suggests that people with chronic pain may be most motivated to self-medicate with substances when they also hold maladaptive pain-related cognitions or are otherwise vulnerable to impulsive behavior when distressed.[64] Indeed, researchers have highlighted several cognitive-affective transdiagnostic constructs (e.g., expectancies, pain-related anxiety, distress intolerance) implicated in the development, maintenance, and exacerbation of bidirectional relations between pain and substance use.[5,9,64]

METHOD

Selection of Relevant Constructs

The constructs of interest in this review were derived from theoretical frameworks of motivation and pain–substance use relations, with a focus on cognitive-affective constructs that are hypothesized mechanisms of the pain–to–substance use pathway and have been implicated as vulnerabilities to multiple psychiatric disorders.[5,9,64] Of particular interest were constructs that are hypothesized causal mediators by which the acute pain experience may serve as a proximal determinant of substance administration, or constructs that may function as moderators (e.g., exacerbating or amplifying existing determinants) or serve to make substance use more salient or incentivized in the context of pain. Modifiable cognitive-affective constructs (briefly described below) were selected as the focus because these constructs have the potential to serve as integrated behavioral targets and to better inform future research and intervention development efforts.

Expectancies, Motives, and Perceived Interrelations

For the current review, expectancies, motives, and perceived interrelations broadly refer to the extent to which people perceive associations between their pain and substance use.[2,5] Whereas

expectancies represent beliefs about what will happen as a result of substance use, motives represent the desired results (i.e., self-reported reasons and valued/desired effects) of substance use.[68] Perceived interrelations further encompass perceptions regarding the co-occurrence and bidirectional effects of pain and substance use.[69]

Pain-Related Anxiety and Pain Catastrophizing

Pain-related anxiety is the tendency to respond to actual or anticipated pain with anxiety or fear, which may motivate avoidance behaviors.[70,71] Similarly, pain catastrophizing reflects the tendency to interpret actual or anticipated pain in an exaggerated manner.[72] The contributions of pain-related anxiety and pain catastrophizing to the onset and maintenance of chronic pain are well recognized.[33] More recently, theoretical models of pain and substance use have included pain-related anxiety and pain catastrophizing as transdiagnostic factors that are also relevant to multiple substance-related outcomes (e.g., craving, heaviness of use, cessation) among people with chronic pain.[5,9,73–75]

Anxiety Sensitivity

Anxiety sensitivity (defined as fear of the potential negative consequences related to anxiety-related symptoms and sensations)[76] is another transdiagnostic factor that is likely relevant to pain–substance use reciprocity.[9] Research has demonstrated independent, positive associations between anxiety sensitivity and heavy/problematic substance use[77,78] and greater pain impairment/persistence.[79–81] There is also evidence that greater anxiety sensitivity may contribute indirectly to the association between pain and poorer outcomes related to substance use and health.[82]

Distress Intolerance

Distress intolerance also may contribute to pain and substance use reciprocity. Research has consistently demonstrated positive associations between distress intolerance (defined as the perceived inability to tolerate negative emotional and/or other aversive states),[83] substance addiction, and poorer cessation/treatment outcomes, including drug and alcohol treatment dropout and substance use relapse.[84,85] There is also evidence that levels of distress intolerance may be higher among individuals with co-occurring pain (vs. no pain in the past month)[86] and that individuals with high distress intolerance are more likely to endorse substance coping motives.[87–89]

Search Strategy and Study Selection

Literature searches were conducted in PubMed, PsycINFO, and Embase using the terms alcohol drinking OR alcohol-related disorders OR analgesics, opioid OR opioid-related disorders; pain; and expectancies OR motives OR perceived interrelations OR negative affect OR pain-related anxiety OR catastrophizing OR anxiety sensitivity OR distress intolerance. All search criteria were limited to human species and peer-reviewed journals published in English before December 2020. Searches yielded 124 unique records after duplicates were removed. Given this review's focus on the pain–to–substance use pathway, the authors sought to identify studies that examined alcohol/opioid criterion variables in relation to at least one of the selected constructs. They included studies that utilized pain-related predictor variables (e.g., pain intensity) or were conducted among relevant pain populations (e.g., chronic pain, persons living with HIV). Studies conducted among healthy, non–treatment-seeking samples were included only if (a) the sample was necessary to answer a pain-related research question (e.g., laboratory experimental pain studies that require healthy participants), and (b) the study included at least one other variable of interest. Primary reasons for exclusion were (a) it was not a behavioral study of the pain–to–substance use pathway (81 studies) or not a relevant population (32 studies).

RESULTS

Twenty-five studies were identified for inclusion (see Table 1).

Table 1 References Identified in Literature Search ($N = 25$), by Variable of Interest

Reference	Author	Year	Design	Outcome(s)
Expectancies, Motives, and Perceived Interrelations				
90	Palfai et al.	2019	Cross-sectional	Alcohol Use
91	Nieto et al.	2020	Cross-sectional	Alcohol Use
92	LaRowe, Maisto, & Ditre	2021	Cross-sectional	Alcohol Use
Negative Affect				
93	Moskal et al.	2018	Experimental	Alcohol Use
94	Witkiewitz et al.	2015	Longitudinal	Alcohol Use
95	Carpenter et al.	2019	EMA*	Opioid Use
96	Finan et al.	2018	Daily Diary	Opioid Use
Pain-Related Anxiety				
73	Zale et al.	2019	Cross-sectional	Alcohol Use
97	Rogers et al.	2018	Cross-sectional	Opioid Use
98	Rogers et al.	2020	Cross-sectional	Opioid Use
99	Rogers et al.	2020	Cross-sectional	Opioid Use
100	LaRowe et al.	2018	Cross-sectional	Opioid Use
111	LaRowe et al.	2020	Cross-sectional	Alcohol Opioid Co-Use
Pain Catastrophizing				
91	Nieto et al.	2020	Cross-sectional	Alcohol Use
101	Lee et al.	2020	Cross-sectional	Opioid Use
96	Finan et al.	2018	EMA*	Opioid Use
102	Martel et al.	2014	Cross-sectional	Opioid Use
103	Arteta et al.	2016	Cross-sectional	Opioid Use
104	Martel et al.	2013	Cross-sectional	Opioid Use
112	Votaw et al.	2020	Cross-sectional	Alcohol Use, Opioid Use
Anxiety Sensitivity				
105†	Rogers et al.	2019	Cross-sectional	Opioid Use
106†	Smit et al.	2020	Cross-sectional	Opioid Use
78†	Rogers et al.	2019	Cross-sectional	Opioid Use
107†	Rogers et al.	2020	Cross-sectional	Opioid Use
108	Rogers et al.	2020	Cross-sectional	Opioid Use
Distress Intolerance				
110	McHugh et al.	2014	Cross-sectional	Opioid Use

* EMA, ecological momentary assessment.

† Studies were drawn from the same sample.

Pain as a Motivator of Alcohol Use

Expectancies, motives, and perceived interrelations

Initial qualitative and cross-sectional evidence suggest that people with chronic pain hold unique cognitions about how pain and alcohol use are related. First, a qualitative study of 10 people living with HIV who had chronic pain and reported heavy drinking (i.e., more than four or five drinks on one occasion or more than seven to 14 drinks per week for women/men) provided evidence that alcohol may be seen by people with chronic pain as a primary means of coping with both pain and pain-related distress.[90] A theme emerged in which alcohol was perceived by the participants to be a "harmless alternative" to prescription opioids for pain management. Among a sample of patients seeking treatment for AUD ($N = 128$), high-intensity pain ratings (vs. no or low-intensity pain) were associated with a greater number of self-reported drinks per day and higher alcohol craving, and participants with high-intensity pain were more likely to report normalizing motives for drinking (i.e., "to feel normal").[91] Finally, researchers recently developed and validated the Expectancies for Alcohol Analgesia measure, which assesses perceived likelihood of pain relief from drinking. In a sample of 273 people who reported chronic pain and current alcohol use, expectancies for analgesia were associated with reporting greater frequency and quantity of alcohol use and identifying coping as a motive for drinking.[92]

Negative affect

Consistent with the larger substance use literature and theoretical conceptualizations of negative affect as a primary motivator of substance use behavior,[3,5,64] there is experimental and observational evidence that the experience of pain, by eliciting negative affect, is a proximal determinant of alcohol use. Laboratory models of human pain utilize standardized noxious stimuli that attempt to approximate features of clinical pain conditions (e.g., neuropathic pain, musculoskeletal pain), reliably elicit sensory pain and subjective distress, and have successfully been used to investigate causal associations between pain and tobacco smoking.[60,61] A recent laboratory experiment conducted among hazardous drinkers further provides causal evidence that pain increases motivation to drink alcohol.[93] Specifically, participants randomized to laboratory pain induction (vs. no pain) reported greater negative affect, which in turn was associated with a greater urge and intention to drink. Prospective evidence derived from two multisite clinical trials for AUD (in the United States and the United Kingdom) provides further evidence that negative affect is a determinant of drinking in the context of pain.[94] Across both samples ($N = 2,125$), pain severity at the end of treatment predicted frequency and quantity of alcohol use at long-term (9- to 12-month) follow-up. Moreover, pain predicted increased negative affect, which mediated the effect of pain on drinking outcomes.

Pain-related anxiety

Although several studies have implicated pain-related anxiety in associations between pain and other substances (e.g., tobacco, cannabis),[5] the search for this review returned only one study that examined associations between pain-related anxiety and alcohol use. In an online survey of 234 adults with chronic pain, pain-related anxiety was positively associated with alcohol-related consequences (e.g., injuries from drinking, blackouts) and impairment in functioning due to alcohol use (i.e., needing a drink in the morning, inability to stop drinking once started, and failure to fulfill obligations due to drinking).[73] Moderation analyses further revealed that associations between pain-related anxiety and drinking were significant among men, but not women.

Pain catastrophizing

Like pain-related anxiety, pain catastrophizing has been widely studied in relation to tobacco smoking and cannabis use.[5,64] However, the authors identified only one study that tested associations between pain catastrophizing and alcohol use outcomes. That study, which tested associations between pain and alcohol consumption and motives among 128

patients seeking AUD treatment, also examined pain catastrophizing as a predictor variable of all outcomes.[91] Results indicated that pain catastrophizing was associated with greater alcohol craving, AUD symptoms, and normalizing drinking motives, regardless of pain intensity.

Pain as a Motivator of Prescription Opioid Use

Negative affect

Initial evidence suggests that negative affect mediates proximal associations between pain and prescription opioid use, similar to alcohol use. Although the search did not return any experiments that tested causal association between pain and opioid use in humans, prospective studies that utilized repeated assessments provide evidence for the effects of pain on prescription opioid use via negative affect. First, real-time ecological momentary assessment among 34 patients on long-term opioid therapy for chronic pain indicated that, over the 2-week assessment period (2,285 total observations), patients were more likely to report opioid use during occasions of increased pain, and they consumed higher doses when pain was accompanied by increased negative affect.[95] Similar results were observed in a daily diary study of patients with pain due to sickle cell disease who were prescribed opioids ($N = 45$). Over the 90-day assessment period, greater levels of pain and negative affect were individually associated with use of opioids at higher doses during the same day, although negative affect was not statistically tested as a mediator.[96]

Pain-related anxiety

Results from several cross-sectional studies suggest that pain-related anxiety is associated with multiple indices of prescription opioid misuse among people with chronic pain. First, in an online survey of young adults ($N = 256$) who endorsed moderate to severe past-month pain, greater pain-related anxiety was associated with increased likelihood of self-reported addiction to opioids, history of family concern about opioid use, past use of opioid detoxification, and more opioid-related problems.[97] Similarly, a study of 164 adults with obesity and chronic pain found that pain-related anxiety was associated with opioid misuse.[98] In a cross-sectional survey of nearly 400 adults with chronic pain, pain-related anxiety was identified as a statistical mediator of associations between pain severity and opioid misuse.[99] Finally, in a clinical sample of 61 smokers of tobacco cigarettes living with HIV and recruited from an infectious disease clinic, higher levels of pain-related anxiety were associated with current opioid misuse among men, but not women.[100]

Pain catastrophizing

Several studies conducted among treatment-seeking chronic pain samples consistently demonstrated positive associations between pain catastrophizing and prescription opioid use. First, among a sample of 51 patients with chronic pain, two facets of pain catastrophizing were positively associated with higher scores on a measure of risk for opioid misuse.[101] Specifically, rumination (i.e., the tendency to have difficulty disengaging from pain-related cognitions) and magnification (i.e., the tendency to magnify perceptions of threat) were each individually associated with risk of opioid misuse. There was also evidence that pain catastrophizing was associated with more frequent cravings for opioids, regardless of pain intensity.[102] Similarly, a daily diary study revealed that people with chronic pain maintained on opioid therapy used higher dosages of their prescription opioids on days in which self-reported catastrophizing was higher, even when pain was low.[96] In addition, two cross-sectional studies conducted among patients maintained on opioid therapy for chronic pain demonstrated evidence of negative affect as a statistical mediator of associations between pain catastrophizing and opioid misuse.[103,104]

Anxiety sensitivity

Among an online sample of 429 adults who self-reported moderate to severe chronic pain and prescription opioid use, anxiety sensitivity mediated associations between greater

pain intensity and opioid misuse and OUD symptoms[105,106] and was associated with greater likelihood of endorsing use of opioid medications "to get high."[78] In the same sample, models of indirect effects indicated that negative affect was associated with opioid misuse through anxiety sensitivity.[107] Interestingly, when the model was run in reverse, equal statistical support was observed for an indirect effect of anxiety sensitivity via negative affect, suggesting that prescription opioid misuse may occur in the context of a complex interplay between negative affect and anxiety sensitivity. Finally, data derived from an online sample of nearly 300 adults who reported chronic low back pain indicated that anxiety sensitivity may have an indirect effect on risk of opioid misuse through greater coping and pain management motives for prescription opioid use.[108]

Distress intolerance

Distress intolerance previously has been associated with tobacco and cannabis use among people with chronic pain and has been implicated in heavy drinking and alcohol-related problems in healthy populations.[5,109] However, only one cross-sectional study that investigated associations between distress intolerance and prescription opioid use was identified. Among a sample of 39 patients at a pain management clinic who were prescribed opioids, greater levels of distress intolerance were associated with greater scores on a measure of opioid misuse risk, even after statistical analyses controlled for pain intensity.[110]

Pain as a Motivator of Alcohol and Opioid Co-Use

Although co-use of alcohol and opioids has potentially dire health consequences, this review identified only one study that directly examined alcohol and prescription opioid co-use in the context of chronic pain. In an online sample of 1,812 adults with chronic low back pain, 12% endorsed use of both alcohol and prescription opioids (co-use) and 3% met cut-offs for both hazardous drinking and opioid misuse in the past month (i.e., concurrent use).[111] Pain-related anxiety was individually associated with hazardous alcohol use, opioid misuse, and likelihood of alcohol and opioid co-use. Moreover, every 1-point increase in pain-related anxiety was associated with a 4% increase in likelihood of concurrent hazardous drinking and opioid misuse. Another recently published study, which examined polysubstance use (defined as use of more than one substance in the month before treatment) among a subsample of 236 people receiving inpatient treatment for AUD or OUD who reported chronic pain, showed that the two most commonly reported substances were alcohol and prescription opioids.[112] Separate statistical models further indicated that pain-related interference with functioning was associated with a greater number of substances used among men (but not women) and among people with AUD (but not people with OUD). No associations were observed between pain catastrophizing and number of substances used.

DISCUSSION

Prior research has demonstrated that pain motivates substance use and may contribute to the maintenance of addiction.[5,6,64] The purpose of the current review was to examine modifiable cognitive-affective factors that may be associated with motivation to use alcohol and prescription opioids in the context of pain. These constructs include key mechanisms in motivational models of both substance use and pain (negative affect, expectancies/motives)[2,113] and factors in the reciprocal model of pain and substance abuse (pain-related anxiety, pain catastrophizing, distress intolerance, and anxiety sensitivity) that may increase vulnerability to both conditions.[5,9,64] Consistent with a reciprocal model of pain and substance use, this review provides evidence that pain is a proximal determinant of alcohol use[114] and opioid use,[95] even among individuals without chronic pain.[114] The review further observed consistent evidence that people with chronic pain are motivated to use alcohol and prescription opioids in response to negative affect[94,95] and maladaptive pain-related cognitions

(e.g., catastrophic thinking).[91,100] Finally, this review found initial evidence suggesting that difficulty managing aversive internal states is associated with risk for opioid misuse,[110] and that people with chronic pain hold unique motives and perceptions about how their pain and drinking are interrelated.[90]

Motivational models of both pain and substance use highlight the role of negative affect as a primary determinant of escape/avoidance behaviors, and negative reinforcement is likely a key mechanism by which pain motivates and ultimately maintains substance use.[5,64] The current review provides some support for this perspective, with experimental and real-time evidence from three studies indicating that negative affect mediates associations between the experience of pain and acute bouts of alcohol and prescription opioid use.[93,95,96] The authors also reviewed three studies that provided cross-sectional evidence of covariation between negative affect and transdiagnostic vulnerability factors, such that negative affect was a statistical mediator of associations between opioid misuse and both pain catastrophizing[103,104] and anxiety sensitivity.[107] Initial evidence from one study further suggests that difficulty with tolerating negative affect (i.e., distress intolerance) is associated with opioid misuse.[110] Taken together, these findings lend support to the notion that people may experience greater motivation to use alcohol and prescription opioids during heightened states of acute and extended pain affect, and that such effects may be amplified in the context of transdiagnostic vulnerability factors that exacerbate (e.g., pain catastrophizing) or diminish capacity for coping with (e.g., distress intolerance) negative affect.

Pain-related anxiety and pain catastrophizing are both thought to motivate maladaptive attempts to avoid or alleviate pain.[115] Consistent with evidence that both prescription opioids and alcohol have acute analgesic effects, the current review provides initial evidence that people who experience chronic pain may view drinking alcohol as a viable approach to pain management[90] or hold expectancies for pain relief from drinking.[92] The review also observed consistent evidence across several studies that pain-related anxiety and pain catastrophizing are associated with alcohol and opioid use among people with chronic pain.[73,96,102,111] Maladaptive cognitive-affective responses to pain may activate escape/avoidance processes, leading to use of alcohol and/or opioids. These observations are in line with conceptual models of pain and substance use and further support consideration of pain-related cognitions as potentially key transdiagnostic vulnerability factors for alcohol and opioid use.[5,9,64]

Although both alcohol and prescription opioids present health risks when used individually, co-use is associated with increased morbidity and mortality and presents a significant public health threat.[7] Despite the dangers of concurrent use of alcohol and opioids, the authors found only one study that was designed to examine co-use of these substances in the context of pain.[111] Consistent with findings from studies of either substance alone, pain-related anxiety was associated with greater likelihood of misuse of both substances concurrently in an online sample of adults with chronic pain. One potential explanation for this finding is that people with higher levels of pain-related anxiety may view concurrent use as a way to extend or supplement analgesic effects of both substances.[116] Although there is reason to suspect that alcohol-opioid co-use also could be seen as a more potent means of escaping/avoiding negative affect (vs. use of either substance alone), this hypothesis is yet to be tested.

Limitations and Future Directions

Studies included in the current review consistently yielded evidence suggesting that negative affect and other maladaptive cognitive-affective responses to pain and distress may cause alcohol and prescription opioids to take on greater salience in the context of pain. As shown in Table 1, this review identified one experimental study and three prospective studies that lend support regarding temporal precedence; however, the majority of reviewed studies were cross-sectional in nature and thus preclude causal interpretations. Future research would benefit from employing experimental and prospective designs to identify causal relationships and monitor covariation between pain-relevant cognitive-affective

constructs and the use or co-use of alcohol and/or opioids over time. For example, prospective studies may test whether maladaptive responses to pain predict escalation of alcohol and/or opioid use or the development of AUD and/or OUD. Ecological momentary assessment provides a promising avenue for assessment of alcohol and opioid co-use in real time and should be considered as a means of better understanding simultaneous use. Indeed, despite the dangers of being under the influence of alcohol and opioids at the same time, the current review did not identify any studies that have investigated motivation for simultaneous use in relation to this review's constructs of interest. Although dichotomous co-use status (yes/no) provides utility at this early stage of hypothesis testing, co-use should be assessed in greater detail (e.g., frequency, quantity, experience of negative consequences, and temporal proximity of alcohol and prescription opioid ingestion). Particular attention should be paid to identifying patterns of heavy drinking (e.g., frequency, quantity) and prescription opioid use (e.g., high dose, prolonged release, and once-daily formulation) that may increase risk of overdose or other harmful effects.[31,117] Research in this area should include a focus on both overlapping and distinct pharmacologic effects of alcohol and prescription opioids.

Future research also is needed to better understand motivation to use alcohol and prescription opioids in the context of other comorbidities. First, co-use of alcohol and opioids with other substances (e.g., nicotine, cannabis)—particularly those that increase risk for medical consequences and public health burden—should be examined. For example, 22% of opioid-related overdose deaths involve co-use of alcohol, opioids, and benzodiazepines.[118] Up to 23% of chronic pain patients prescribed opioids also hold a concurrent prescription for a benzodiazepine,[119] and risk of opioid-related overdose death among this group is 10 times greater than among those who hold an opioid prescription alone.[120] Anxiolytic properties of benzodiazepines may further encourage use as a method to escape/avoid pain and negative affect. Similar considerations regarding temporal precedence and the need for comprehensive assessment of dynamic substance use patterns should be applied to the study of polysubstance use in the context of pain. This review's transdiagnostic approach, which focuses on vulnerability factors implicated in a range of psychiatric conditions (e.g., depression, post-traumatic stress disorder, anxiety, personality disorders),[121–124] also highlights the complexity and interrelatedness of pain, substance use, and psychiatric comorbidities. Future research should seek to identify additional vulnerability factors that may contribute to or exacerbate relations between pain and use or co-use of alcohol or prescription opioids. For example, separate literatures have shown bidirectional relationships between sleep disturbances (e.g., difficulty falling or staying asleep) and both substance use[125] and pain;[126] researchers should consider a range of behavioral and psychiatric comorbidities when studying alcohol and prescription opioid use in the context of pain. Finally, the majority of included studies focused on a single construct of interest, and additional studies should be conducted to examine interrelations and unique contributions of the constructs examined in this review. Indeed, several studies examined negative affect as a statistical mediator of associations between cognitive-affective factors (i.e., anxiety sensitivity, catastrophizing) and opioid use,[103,104,107] and only one study tested associations between a cognitive-affective variable and self-reported motives for opioid use.[108] Future research is needed to disentangle likely complex and bidirectional associations between this review's variables of interest.

Given the central role of motives and expectancies in motivational models of substance use,[3] the authors were surprised to find a paucity of studies that examined expectancies and motives using validated measures (e.g., the Alcohol Expectancy Questionnaire[127]). Albeit limited, data from this review are in line with the larger literature suggesting that people with chronic pain hold substance-related outcome expectancies for pain relief and coping.[5] Recent validation of the

Expectancies for Alcohol Analgesia scale provides evidence that people with chronic pain can reliably self-report expectancies for pain relief from alcohol and that perceived likelihood of analgesic effects may motivate greater frequency and/or quantity of drinking.[92] In addition to coping, future research is needed to identify other types of motives and expectancies that may motivate substance use in the context of pain. For example, chronic pain often results in decreased social functioning, and researchers have hypothesized that social drinking motives may be particularly salient as a means of reducing pain-related interference in social functioning.[6] Similarly, as chronic pain interferes with occupational, social, and recreational functioning, people who have high levels of pain-related disability often lose access to other positive reinforcers.[4] A key component of incentive motivation models involves weighing the incentive value of substance use against other incentives available in the environment.[2,113] For people with chronic pain, expectancies regarding the positive reinforcing effects (e.g., to feel good or high) may become particularly salient. Future research should assess a range of expectancies and motives (including and beyond coping with pain and direct pain reduction) for alcohol, opioids, and co-use of both substances among people with chronic pain. Future research also would benefit from examining the role of positive affect and positive reinforcement processes in bidirectional alcohol and opioid use processes.

Although only a few included studies examined sex/gender differences, this review did observe initial evidence that pain and pain-related constructs may hold greater motivational salience for alcohol and prescription opioid use among men, relative to women. Future research should investigate reasons why men may be more motivated to use substances in the context of pain. For example, men and women likely experience varying pharmacologic effects of alcohol and opioids as a function of biological sex,[128] and it may be that men derive greater analgesia from use or co-use of alcohol and prescription opioids.[129] Considering gender as a biopsychosocial construct,[130] there is reason to suspect that men and women experience pain and substance use differently. For example, masculine norms may influence which pain-related behaviors are most common or accessible to men,[131] which is consistent with a larger literature indicating that men are more likely than women to cope with pain and anxiety by using externalizing strategies.[132,133] Thus, future studies that consider pharmacokinetic properties of prescription opioids and alcohol should examine sex as a biological variable, and research that investigates psychosocial and behavioral constructs should explore gender differences. Although the findings presented in the current review may have been drawn from cisgender samples, future research should test associations between pain and substance use among transgender and gender minority populations.[134,135] Additionally, despite documented racial/ethnic disparities in the prevalence, treatment, and outcomes of pain-related conditions,[136,137] this review identified only one study that examined the current constructs of interest among racial/ethnic minorities.[97] A recent review of racial and ethnic disparities in chronic pain treatment found that Black patients maintained on long-term opioid medication were more closely monitored for misuse, despite higher rates of opioid misuse and opioid-related overdose deaths observed among Whites.[138] Furthermore, racial and ethnic minorities, including Native Americans, Blacks, and Hispanics, are disproportionately impacted by drinking, including greater alcohol-related problems and reduced access to treatment, compared to Whites.[139] Initial evidence derived from the tobacco literature suggests there may be important racial/ethnic differences in associations between cognitive-affective constructs, pain experience, and substance use.[140] Future research would benefit from examining disparities as a function of social/cultural characteristics, racial/ethnic discrimination, and economic disadvantage, among others.

Finally, this review identified studies that predominantly focused on motivational processes in the context of ongoing substance use. Additional research is needed to better understand whether cognitive-affective and transdiagnostic vulnerability factors are also associated with

motivational processes in the context of substance-related cessation, reduction, and abstinence. For example, several constructs (e.g., pain-related anxiety) previously were associated with relapse to tobacco smoking among people with chronic pain.[75] Although this review identified one study that examined long-term outcomes after participation in treatment for AUD, the authors are not aware of any work that has directly tested these constructs in relation to lapse/relapse trajectories for alcohol and/or prescription opioid use. Consistent with a phase-based approach to treating substance use,[141] research should focus on the full spectrum of substance use outcomes rather than on long-term cessation rates alone. Future research also should seek to identify the extent to which expectancies for pain relief and cognitive-affective responses to pain may be related to motivation to quit or reduce use or co-use of alcohol and prescription opioids, or whether such factors influence differential acceptance of referral to alcohol treatment or to programs for tapering prescription opioid use.

Clinical Implications

Although based on a relatively nascent empirical literature, the current review highlights the importance of assessing pain when treating alcohol and prescription opioid use. Integrated treatments may be especially useful for addressing co-occurring pain and substance use because, relative to traditional approaches (e.g., distinct treatments for individual disorders delivered sequentially), they can be more efficient and cost-effective and can focus on both conditions as treatment targets.[142] The currently reviewed cognitive-affective constructs represent intuitive targets for integrated treatments because of their likely transdiagnostic role in both substance- and pain-related processes across multiple disorders. Indeed, substance use interventions that address transdiagnostic vulnerabilities (e.g., anxiety sensitivity) are well underway,[143,144] and this work should be extended to the domain of alcohol and opioid use or co-use. Several evidence-based techniques have been used to address maladaptive appraisals of pain and negative affect, including cognitive restructuring (i.e., development of balanced, adaptive thought patterns),[145] graded exposure to pain and distress-eliciting stimuli (i.e., to reduce escape/avoidance behaviors),[146,147] and coping skills training (e.g., skills to tolerate distress and pain without substance use).[148–150] In addition, integrated behavioral interventions may be utilized in concert with pharmacotherapy, and several pharmacotherapies that are commonly used for pain management (e.g., gabapentin, bupropion) are under investigation for their utility in the treatment of alcohol use and misuse.[151–153] Future work should consider whether there may be synergistic benefit to including pharmacotherapy in integrated interventions for pain and substance use.

Motivational enhancement interventions that target treatment engagement (e.g., willingness to accept a referral for opioid tapering) and motivation to reduce or abstain from alcohol and opioid use also are needed. Although interventions to increase awareness of opioid overdose risk behaviors have been developed,[154,155] the authors are not aware of any treatments that address alcohol and opioid co-use in the context of pain. An integrated treatment for alcohol and opioid use could focus on increasing knowledge regarding adverse interrelations between pain, alcohol, and opioids; increasing motivation and intention to reduce hazardous alcohol use and misuse of opioid medications; and reducing intentions to co-use alcohol and prescription opioid medications. Consistent with a motivational enhancement approach, an important intervention component would be to make an explicit link between continued alcohol/opioid use and poorer pain outcomes, and to highlight pain-related benefits of cessation (i.e., clinically meaningful improvements in pain and interference have been documented after reducing hazardous drinking[6,156] and opioid tapering[157]).[158] Indeed, recent studies derived from the tobacco literature suggest that chronic pain patients may be motivated to reduce their substance use once they perceive a discrepancy between continued substance use and desired pain outcomes.[159,160]

Finally, personalized feedback interventions (PFIs) are a subset of motivational interventions that

show greater promise for treating co-occurring pain and substance use, and can be delivered via scalable treatment modalities (e.g., computer-delivered interventions, smartphone applications).[161] PFIs motivate behavior change via psychoeducation and presentation of feedback about personal behavior (e.g., risk severity) in normative comparison to others (e.g., from relevant sociodemographic groups).[162,163] Parallel lines of inquiry indicate that PFIs decrease maladaptive cognitive-affective and behavioral responses to pain,[164,165] and that they reduce hazardous drinking as well as progression to and maintenance of AUD.[166,167] Two recent studies from the tobacco literature indicated that a brief, single-session computerized PFI is sufficient to increase knowledge of interrelationships between pain, opioid use, and smoking[168] and motivation/intention to quit smoking.[159] Computerized or smartphone-based PFIs can be integrated efficiently with self-monitoring and momentary assessment tools to provide immediate or same-day personalized feedback or just-in-time adaptive interventions (i.e., delivery of intervention content that has been adapted based on time-varying factors, including the state of vulnerability and receptivity to support), and can be used to increase treatment engagement.[164,169,170] These results provide support regarding the potential of integrated PFI interventions for pain, alcohol, and opioids.

CONCLUSIONS

Chronic pain and use of alcohol and prescription opioids co-occur frequently, and pain is a potent motivator of alcohol and opioid use. People with chronic pain may be motivated to use alcohol and opioids in response to negative affect or in response to expectancies/motives for pain coping. Transdiagnostic vulnerabilities for maladaptive responses to pain (pain-related anxiety, pain catastrophizing) and difficulty managing or tolerating aversive states and negative affect (distress intolerance, anxiety sensitivity) also may motivate alcohol or opioid use in the context of pain. Future research should examine the role of transdiagnostic factors in motivating patterns of alcohol or opioid use or co-use over time (e.g., escalations in frequency/quantity of use, lapse/relapse trajectories). Integrated interventions for alcohol and prescription opioid use that address pain-relevant, cognitive-affective processes (e.g., motivation for escape/avoidance of pain or negative affect) also should be developed and tested.

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Brain Structure and Function in Recovery

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Alcohol use disorder (AUD) commonly is associated with compromise in neurobiological and/or neurobehavioral processes. The severity of this compromise varies across individuals and outcomes, as does the degree to which recovery of function is achieved. This narrative review first summarizes neurobehavioral, neurophysiological, structural, and neurochemical aberrations/deficits that are frequently observed in people with AUD after detoxification. Subsequent sections review improvements across these domains during recovery, taking into account modulators of recovery to the extent permitted. Where appropriate, the discussion includes work integrating outcomes across domains, leveraging the strengths of diverse experimental methods. Interventions to ameliorate neurobiological or neurobehavioral deficits do not constitute a primary objective of this review. However, their consideration is a logical inclusion. Therefore, a limited introduction to existing methods is also presented.

KEY WORDS: alcohol; alcohol use disorder; neurobehavioral deficits; brain structure; neurophysiology; neurochemistry; recovery; neural networks

INTRODUCTION

Alcohol use disorder (AUD) is characterized by dysregulation across a range of neurobiological and/or neurobehavioral domains. Neurobiological aberrations include dysregulated neural activity and patterns of brain activation as well as compromise in gray and white matter. Neurobehavioral aberrations are widespread and evident across diverse neuropsychological domains such as problem-solving, learning, memory, and motor functions. An estimated 50% to 80% of people with AUD demonstrate significant cognitive/behavioral compromise relative to community comparison groups, with a substantive minority (i.e., 30% to 40%)[1] exhibiting sufficient compromise to meet criteria for clinical impairment.[2] Describing alcohol-related impairment is further complicated by the fact that neurobiological (e.g., structural) aberrations and behavioral compromise are not universally related. Importantly, empirical studies demonstrate that both neurobiological and behavioral measures improve substantially after

recovery is initiated, although the trajectories vary and are often incomplete. This narrative review focuses on improvements in brain structure and function and briefly explores opportunities for facilitating these processes. To establish an appropriate context, the article begins with a limited overview of alcohol-related biobehavioral deficits. More comprehensive coverage of alcohol-related impairment is provided in several recent reviews.[3,4]

In discussing recovery, several caveats warrant attention. First, there is a paucity of data from individuals who address their alcohol misuse without seeking formal treatment. Thus, this review is largely limited to outcomes obtained from people who participated in inpatient or intensive outpatient treatment.

Second, the phrase "in recovery" eludes ready definition. The goals of both the individuals with AUD and the treatment programs vary. If a program is abstinence based, the objective is to sustain abstinence after treatment, and an individual is considered "in recovery" as long as they maintain abstinence. If the primary treatment objective is harm reduction or controlled drinking, successful recovery is marked by a reduction in negative consequences, without abstinence as a necessary prerequisite. Consequently, while both people who sustain abstinence and those who successfully navigate harm reduction efforts can be considered "in recovery," their continuing exposure to alcohol may vary significantly. Thus, heterogeneity in continued drinking across studies creates a substantive interpretational challenge, prohibiting broad conclusions regarding the effects of "recovery" on neurobiobehavioral improvement. To address this challenge, studies need to incorporate alternate definitions of "successful" outcomes, perhaps also including neurobiobehavioral improvement as one component. In the extant literature, the majority of reports are derived from treatment-seeking individuals in abstinence-based programs. Nevertheless, rather than relying only on binary outcomes (e.g., relapse vs. sustained abstinence), some investigations, as illustrated in later sections, gather data regarding continuing drinking patterns, providing a more granular consideration of alcohol use across time.

Third, many studies use the phrases "recovery" and "improvement" of function interchangeably. At initial glance, distinguishing these terms seems a matter of semantics. However, as addiction science directs attention to the effectiveness of interventions in enhancing outcomes, the distinction is highly relevant.[2] Conservatively defined, improvement references positive change associated with the passage of time (i.e., time-dependent change) or repeated practice (i.e., practice effects). For example, cognition improves with time after detoxification, even without directed intervention, as well as after repeated testing. The phrase "recovery of function," in contrast, refers to positive change that cannot be accounted for by time or practice. Distinguishing "improvement" from "recovery" requires the inclusion of appropriate comparison data and is particularly relevant when evaluating behavioral outcomes and interventions. In the following sections, the terms are used with attention to this distinction. That said, positive change is a desired outcome, whether or not it meets a strict definition of recovery of function.

Fourth, although the potential influence of individual variables such as age and sex/gender on recovery is widely recognized, it has not been systematically studied, particularly in longitudinal assessments. Therefore, these variables are not discussed in depth here.

BRIEF OVERVIEW OF ALCOHOL-RELATED SEQUELAE

This section provides brief overviews of four broad categories of alcohol-related biobehavioral sequelae: neurobehavior, neurophysiology, brain structure, and neurochemistry.

Neurobehavior

A substantial literature has illustrated that cognitive processes relying heavily on the

prefrontal and frontal cortices (i.e., executive functions such as attention, working memory, problem-solving, inhibition, and flexibility) are susceptible to chronic excessive alcohol consumption.[5] However, alcohol-related deficits are not limited to these domains. Compromise in visual-spatial functions, gait/balance, and new learning/memory is also frequently reported.[6] Taken together, alcohol-related deficits in neuropsychological/behavioral functions often are described as reflecting a mild, generalized brain dysfunction.[2,6] Beyond these traditional neuropsychological characteristics, interest in alcohol-related compromise in key facets of emotion processing and social cognition is increasing. Of particular note are deficits in emotion face processing, interpersonal problem solving, and humor processing,[3,7,8] all of which are critical skills in social, work, and family settings.

Neurophysiology

Brain electrophysiology, as obtained from scalp electrodes, also is affected by chronic alcohol misuse. Studies have revealed dysregulation in the electroencephalogram (EEG), as well as in the amplitudes and/or latencies of electrophysiological components that occur at specific times following stimulus presentation or response (i.e., event-related potentials [ERPs]).[9,10] Importantly, both ERP components that occur earlier after stimulus presentation (i.e., exogenous components) and reflect sensory processes and components that occur later (i.e., endogenous components) and reflect cognitive processes are sensitive to chronic excessive alcohol use. This demonstrates alcohol's impact on the temporal dynamics of both sensory and cognitive processes.[7,9,10] A growing body of alcohol research has focused on performance monitoring, which entails ongoing monitoring of response accuracy in the context of changing demands. A common variable studied in these protocols is the error-related negativity (ERN), which is observed after the subject commits an error while completing speeded response tasks.[11] Accurately detecting errors is essential for adaptive behavior. Thus, findings of aberrant ERN amplitudes in people with AUD[12] suggest compromise in the biobehavioral dynamics underlying adaptive behavior.

Repetitive patterns of neural activity (i.e., neural oscillatory activity) and the amount of brain activity in certain frequency bands (i.e., EEG power) reflect a coordinated (i.e., synchronous) neuronal discharge that can be examined as a function of both time and frequency. EEG power can be examined in either a resting state or during specific sensory or cognitive events. In the latter case, the activity is referred to as event-related oscillations. AUD is associated with alterations in both types of measures, demonstrating widespread dysregulation in the temporal dynamics of neural processes.[10]

Brain Structure

People with AUD frequently exhibit volumetric loss in gray and white matter, as well as ventricular expansion in both the cerebrum and cerebellum.[13,14] Data regarding sex differences are mixed, with some studies suggesting that women are more susceptible than men to alcohol's effects while other studies show either no pattern or the opposite pattern.[15] Higher vulnerability also has been reported with increasing age, especially in frontal brain areas.[16] Beyond reduced brain volumes, studies have shown compromised white matter integrity,[17,18] with indications of age interactions.[19]

Dysregulation in brain network activity and connectivity also frequently occurs.[20] Although the default mode network[21] has received greatest attention, other networks are impacted as well, including the executive control, salience, and reward networks.[22,23] Finally, associations may exist between structural compromise and neurobehavioral measures. For example, Pandey and colleagues[18] found significant relationships between white matter fractional anisotropy measures and neuropsychological performance.

Neurochemistry

Several studies have demonstrated that neurochemistry is also disrupted in AUD.[24,25] Using proton magnetic resonance imaging, the most frequently reported findings indicate lower levels of the neuronal metabolite *N*-acetylaspartate (NAA), as well as of choline-containing compounds (Cho) and creatine metabolites (Cr). Findings are mixed regarding alcohol's effects on the glial metabolite myo-inositol, and complex outcomes are associated with measures of the neurotransmitters glutamate and gamma-aminobutyric acid (GABA).[26]

Summary

Although they do not occur in all people with AUD, alcohol-related deficits in neurobehavior, neurophysiology, brain structure, and neurochemistry constitute significant individual and public health concerns. Deficits across the four domains are incompletely correlated and often fall short of criteria for clinical impairment. Nevertheless, they can impact treatment engagement, post-treatment adaptation, and relapse.[27-30] Thus, clarifying recovery trajectories, identifying relevant individual and confounding variables, and determining effective interventions must be research priorities.

EFFECTS OF RECOVERY

Fortunately, with continuing recovery, neurobiobehavioral impairment can improve. The following sections discuss neurobehavioral, neurophysiological, structural, and neurochemical recovery in more detail.

Neurobehavioral Change in Recovery

Investigations suggest that substantial improvements in neurobehavioral functions occur during the first 4 to 8 weeks of abstinence, followed by more modest mid-term (i.e., approximately 1 year) gains. Verbal skills typically improve most quickly, while other domains, although improved, may remain compromised for several months to years.[31] Longitudinal studies also found substantive differences in change trajectories across domains, supporting the general conclusions derived from cross-sectional comparisons of subgroups of people with AUD who differed in abstinence length.[4] Petit and colleagues[32] recently investigated the effects of abstinence on alcohol-related working memory and inhibitory control deficits. By the third week of abstinence, working memory function was indistinguishable between the AUD and control groups, whereas inhibitory control deficits remained. Employing a similar 3-week test interval, Cordovil De Sousa Uva and colleagues[33] also observed deficits in inhibitory control and executive functions at initial testing, but noted no improvements at retest for either function. Not surprisingly, recovery across these three overarching domains appears to be greatest with abstinence.[27,34-36] However, it is noteworthy that some data suggest that low or moderate posttreatment drinking may not preclude improvement.[29]

Studies of improvement in cerebellum-linked behaviors such as gait, balance, and postural sway have produced mixed results. Fein and Greenstein[37] examined these functions in a longitudinal study of people with AUD, with a baseline assessment at 6 to 15 weeks of abstinence and follow-up 4 to 16 months later. Performance was compared with healthy control subjects who also were tested twice. The AUD group performed more poorly than the control group at both assessments and demonstrated no improvement across time. The investigators note that the analyses would have missed improvement occurring before the first assessment (i.e., an average of about 10 weeks of abstinence). However, persistence of deficits in cerebellar functions also has been demonstrated in other studies and in both men and women.[38] To date, most studies on the recovery of alcohol effects on the cerebellum have been restricted to measures of stability and related outcomes. This focus is expected to expand with increasing appreciation of the cerebellum's role in extended brain networks.[39,40]

Research regarding initial deficits as well as recovery in social cognition is limited and has yielded mixed results,[3] but recent work provides encouragement. For example, Erol and colleagues[41] observed improvements in emotion identification accuracy, with performance in people with AUD at 3 months of abstinence equivalent to that of control subjects. It is possible that improvement in emotion processing and social cognition may require more time than do more commonly investigated cognitive functions.

One limitation of these studies is that AUD-focused longitudinal examinations often assess participants only at two time points and typically within a relatively narrow time frame to minimize participant attrition and ensure study feasibility. This practice significantly constrains understanding of continued recovery and limits estimations of within-person heterogeneity, minimizing the opportunity to identify differential predictors and trajectories at the level of the individual. A study by Bates and colleagues[42] provides a notable exception, revealing marked within-person heterogeneity and illustrating substantive challenges in predicting recovery trajectories.

Nicotine use, particularly chronic smoking, is common in people seeking treatment for AUD. Several studies have examined its potential role in exacerbating alcohol-related deficits. Durazzo and colleagues[34] compared recovery trajectories across an 8-month assessment period in active smokers and nonsmokers with similar initial deficits. Whereas the nonsmokers demonstrated recovery of cognitive function, the active smokers retained measurable deficits on multiple measures. Age played a significant role in this relationship, with older active smokers evincing the least improvement over time.[43] In a recent follow-up study, Durazzo and Meyerhoff[44] compared people with AUD who were either never smokers (nvsALC), former smokers (fsALC), or active smokers (asALC) with a healthy control group. All participants were tested twice: The AUD groups were assessed at about 30 days of abstinence and again at about 8 months of sustained abstinence, and the control group was tested and retested at a similar interval. In contrast to earlier work focusing on learning/memory,[34] the researchers administered a more comprehensive battery. Smoking status accounted for differential recovery across all neurocognitive domains, including executive functions (see Figure 1), with active smokers exhibiting the least recovery.

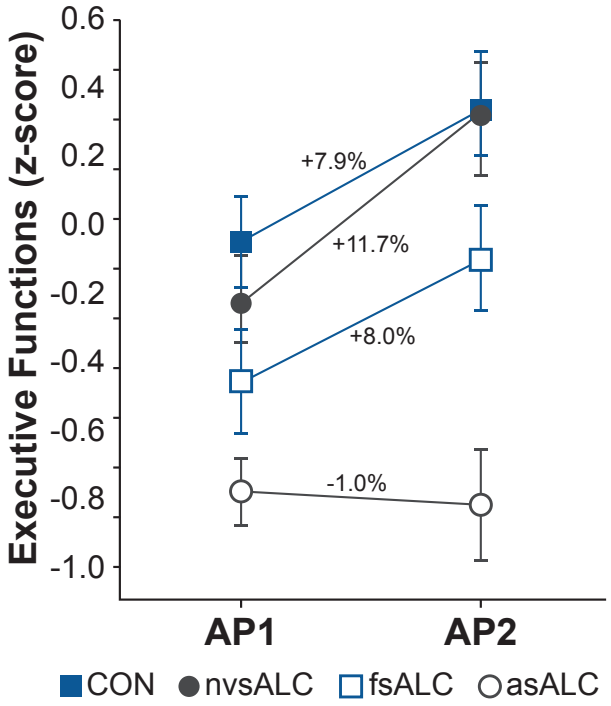

Figure 1 Effect of smoking status on recovery of executive functions during abstinence. Over an 8-month post-treatment period, individuals with alcohol use disorder (AUD) who never smoked evinced greater improvement in executive functions (as indicated by z-score) relative to all other groups. Active smokers showed no improvement between assessments, remaining inferior to controls and people who never smoked. The slight increase in the control group could be expected based on practice effects. *Note:* AP1: 33 ± 9 days abstinent; AP2: 232 ± 56 days abstinent; CON: never-smoking controls; nvsALC: never-smoking individuals with AUD; fsALC: former smokers with AUD; asALC: active smokers with AUD. *Source:* Durazzo and Meyerhoff, 2020.[44] Reprinted with permission from Elsevier Inc.

Neurophysiological Change in Recovery

The degree to which brain electrophysiology improves with abstinence is variable and influenced by family history of AUD. For example, seminal studies showed that components of early sensory potentials, such as the brainstem auditory evoked response, exhibited improved morphology, shortened conduction times, and shorter latencies at 4 months of abstinence than at 1 month of abstinence.[9] In contrast, amplitudes for the P3—a later component associated with context (target) processing, cognitive control, and feedback processing—remained dampened. Importantly, a family history of AUD accounted for much of the variability in P3 amplitude. Similar observations across numerous studies have led to the proposal that P3 aberrations, particularly blunted P3 amplitudes, constitute a possible AUD endophenotype.[10,45,46]

Using a cross-sectional design, Fein and colleagues[47] investigated the effect of abstinence on neurobiological variables, comparing individuals with AUD who were long-term abstinent (abstinence ≥ 6 months, mean abstinence > 6 years) and community controls. The investigators examined the P160—an ERP component with demonstrated sensitivity to face processing and reaction time—using an emotional face expression task. In this task, individuals must select the emotion expressed by individually presented faces. The control task required identifying a neutral face as either male or female. Compared with the community controls, the long-term abstinent group demonstrated longer P160 latencies on both tasks and slower reaction times on the emotional face expression task only. The P160 effects remained significant even after accounting for reaction-time differences. In contrast to other work,[9,10] family history of AUD did not influence outcomes in the current study. Also, no significant sex by group interactions were observed, a finding contrary to the common conclusion that men and women are differentially vulnerable.

Several studies have used resting state synchrony (RSS) in studies of recovery. RSS reflects the level of synchrony in activation and/or deactivation within or across brain areas when an individual is not actively engaged in a neurocognitive task, i.e., at rest. Using RSS, Camchong and colleagues[35,36] examined differences between short-term (mean = 73 days) and long-term (mean = 7.9 years) abstinence as reflected in activation patterns within the executive control and reward processing networks. They found that, when compared to community controls and individuals with short-term abstinence, individuals with long-term abstinence displayed significantly lower levels of RSS in the reward processing network than did either the short-term abstinent or community control groups. Individuals who had achieved short-term abstinence fell intermediate to the community and long-term participants, but did not differ significantly from the control participants. Longer abstinence was also associated with higher levels of RSS in the executive control network, although group comparisons indicated that only the contrast between the long-term and community groups was statistically different.

Alterations in processes underlying intentional behavior likely contribute to long-term outcomes. As previously described, the ERN is an indicator of effective performance monitoring. A recent cross-sectional study examined the ERN in (a) actively drinking, non–treatment-seeking people with AUD; (b) individuals meeting criteria for remitted AUD using clinical criteria assessing drinking consequences and which do not require abstinence (mean = 2.8 years in remission); (c) individuals with a family history of AUD, but not having an AUD themselves; (d) people without histories of AUD who met criteria for non-psychotic disorders such as anxiety or depression; and (e) healthy controls.[12] In contrast to earlier reports indicating that AUD was associated with higher ERN amplitudes,[48] the actively drinking AUD group in this study produced significantly lower ERN amplitudes than each of the other groups, which did not differ among themselves (see Figure 2). Interestingly, there were no group differences in accuracy rate or reaction times for errors. Also, the study found no effect of a family history in the AUD groups, although prior work by Fein and Chang[49] had indicated that an increased family-history density in people with AUD was associated with lower

ERN amplitude. Regardless of the direction of the alcohol effect or the possible role of a family history of AUD, these data implicate dysregulation in neural activity in detecting behavioral errors, which is a critical aspect of effective intentional behavior.

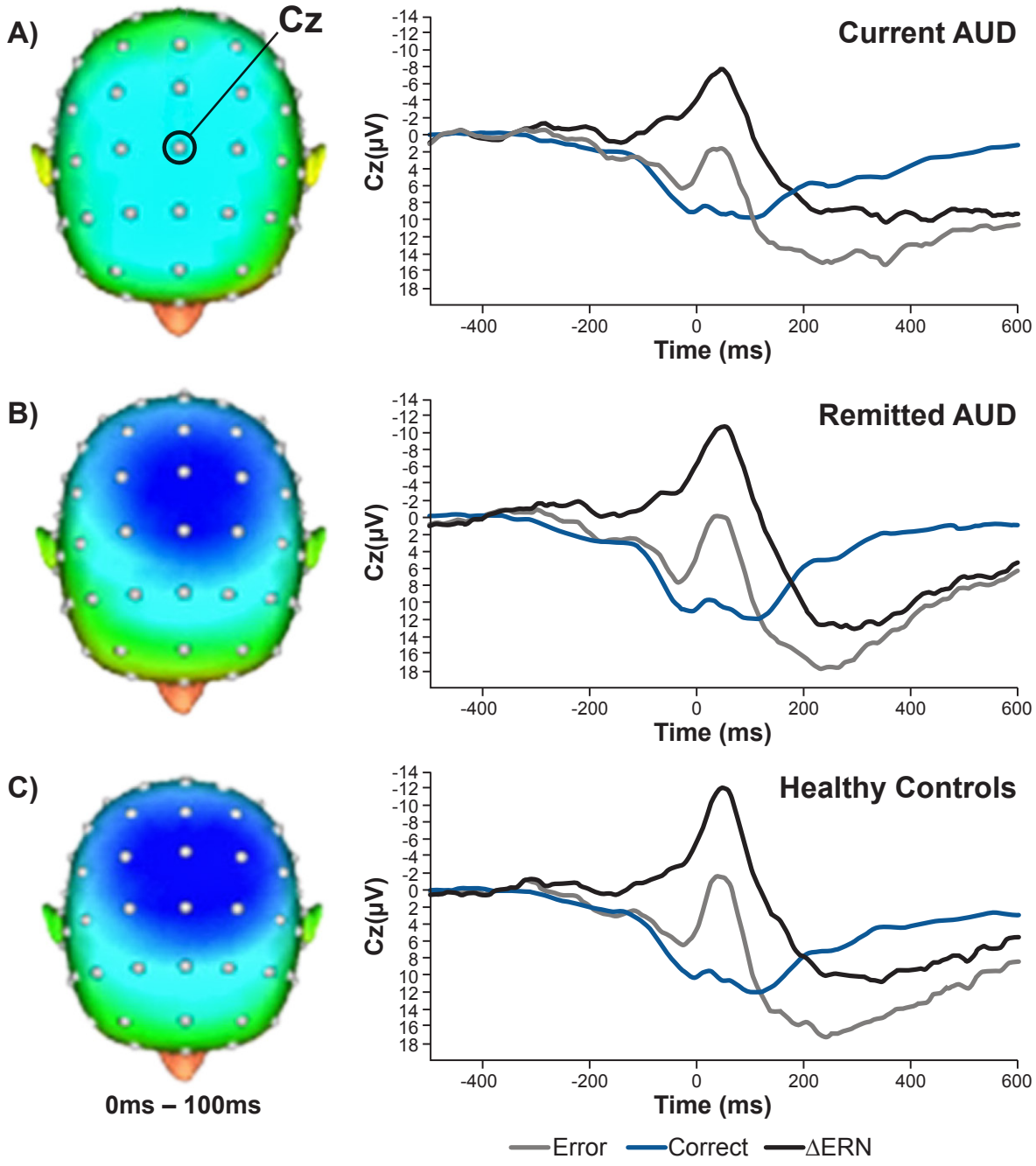

Figure 2 Error-related neural activity among (A) people with current AUD, (B) people with remitted AUD, and (C) healthy controls. (Left) Topographic maps of neural activity (error minus correct). (Right) Response-locked event-related potential waveforms for correct trials, error trials, and difference waves (error-related negativity; ΔERN). Current AUD was associated with greater blunting of the ΔERN amplitude relative to both healthy controls (Cohen's $d = 0.52$) and individuals with remitted AUD (Cohen's $d = 0.37$). Individuals with remitted AUD did not differ from healthy controls. Cz: electrode located at the central midline position; ms, milliseconds. *Source:* Gorka et al., 2019.[12] Reprinted with permission from Elsevier Inc.

Structural Change in Recovery

Demirakca and colleagues[50] studied change in gray and white matter in treatment-seeking men and women between 5 weeks and 3 months of post-treatment abstinence. They found a significant reduction in cerebral spinal fluid (CSF), an indicator of ventricular enlargement and significant increases in gray matter volume, particularly in the insula and cingulate gyrus, for participants who sustained abstinence over the interim period. In contrast, participants who used alcohol, regardless of the amount, demonstrated no change. Unfortunately, the sample size was insufficient to address potential sex differences. Another study compared imaging analyses of treatment-seeking individuals with AUD and healthy controls on day 1 and day 14 of treatment.[51] The treatment group showed significant, but incomplete, recovery in gray matter volume even across the limited time frame, with the cingulate gyrus, temporal gyrus, parietal lobule, cerebellum, and precuneus exhibiting greater improvement than other areas examined. A preliminary examination of sex differences revealed no sex by group interactions, suggesting the absence of sex differences in the trajectory of this measure of brain recovery.

Another longitudinal study examined structural changes over a 6-month period.[29] Rather than using a binary classification of outcomes (i.e., sustained abstinence vs. return to alcohol use), the investigators quantified alcohol use across the study period. The analyses indicated an inverse relationship between consumption across the 6 months and volume increases in diverse brain regions, including the cerebellar vermis, fusiform gyrus, striatum, and cingulate gyrus. The pattern of this association suggested that measurable brain volume improvement may be observed with low to moderate alcohol use after treatment, at least over this 6-month period. However, the small sample size dictates caution in broad generalization.

Employing longitudinal assessments of their sample, Meyerhoff, Durazzo, and colleagues conducted a series of analyses based on longitudinal assessment of individuals with AUD to address recovery trajectories. Imaging sessions at 1 week, 1 month, and 7.5 months of sustained abstinence found substantive volume increases in the frontal, parietal, and occipital lobes as well as increases in the thalamus and cerebellum and a reduction in ventricular volumes.[52] The recovery trajectories differed between gray and white matter. Regional lobar white matter showed a linear increase across the assessment period. In contrast, regional gray matter showed a nonlinear pattern, with most of the change occurring in the interval between 1 week and 1 month. Even with these increases, the AUD group had lower gray matter volumes than control subjects at the final assessment, with the exception of the frontal lobe. The analyses also identified an interaction of age and smoking, such that with increasing age, the recovery of total cortical and frontal gray matter in individuals who smoked was reduced compared with those who did not smoke. This pattern was consistent with the observed behavioral recovery. The sample was composed primarily of men (88% to 93%, depending on group), precluding study of sex differences.

The researchers also used these data to examine differences between the AUD group and the control group, as well as over time, in brain regions representing core components of the executive control, salience, and emotion networks. These included the dorsal lateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC), the orbitofrontal cortex (OFC), insula, amygdala, and hippocampus. The analyses determined that amygdala volumes were not compromised at any point in people with AUD. Also, at the final assessment, the volumes of the ACC, DLPFC, OFC, and insula were equivalent in the AUD and control groups, whereas hippocampal volume remained lower in the AUD group.[53]

A third analysis by this research group explored associations between initial compromise, improvement across time, and treatment outcomes. Comparisons of people with AUD who sustained abstinence versus those who relapsed over the 12 months after treatment showed differences between controls and the two groups even at the initial assessment. People with AUD who eventually relapsed had smaller volumes in three times the number of regions (15/20) as did those who sustained abstinence (5/20). Moreover, among the relapse

group, greater gray matter increases during the early weeks of sobriety were associated with longer delays to relapse.[28]

Mueller and Meyerhoff[27] also assessed loss in gray matter and gray matter connectivity within the extended brain reward system—that is, OFC, DLPFC, ACC, insula, striatum, thalami, hippocampi, and amygdala—and its connections with other networks. In longitudinal comparisons at about 1 month abstinent and 3 months later, they found significant resolution in individuals who had sustained abstinence while measures for those who had relapsed remained essentially unchanged (see Figure 3).

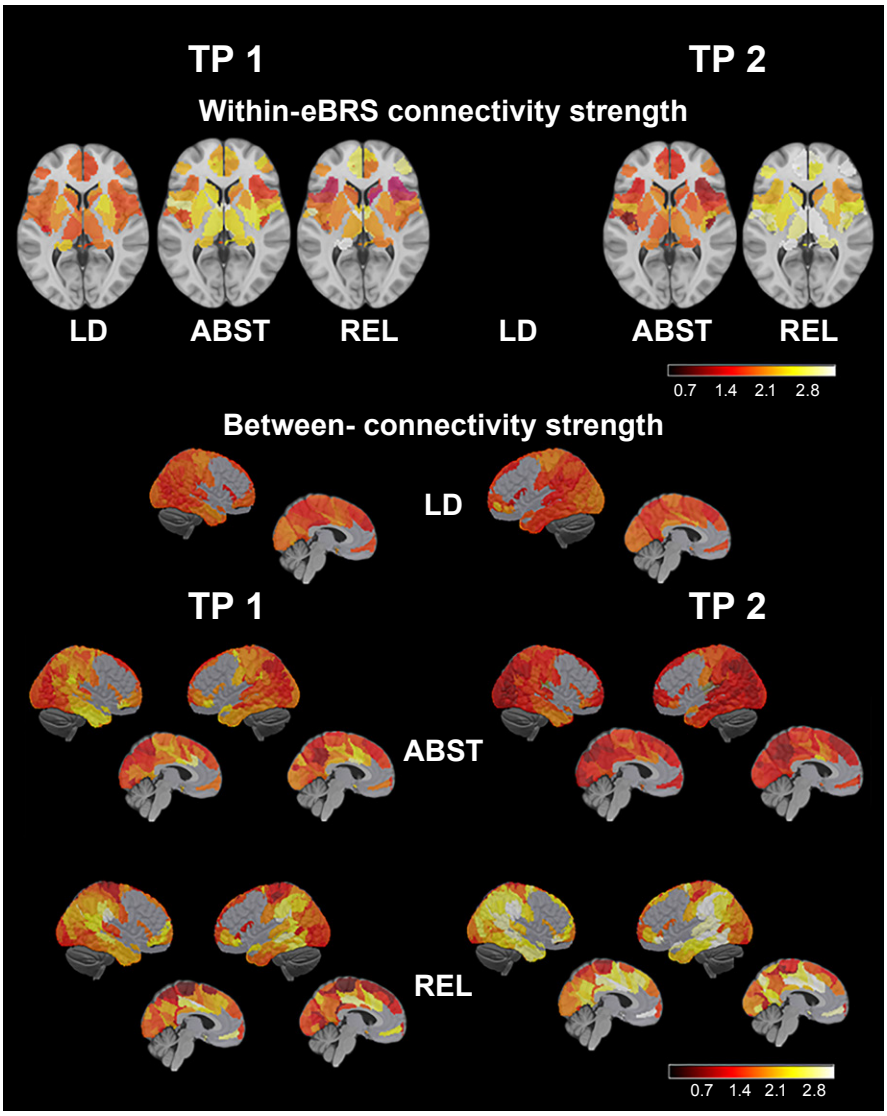

Figure 3 Within-network and between-networks gray matter connectivity. (Top) Images on the left show within-extended brain reward system (eBRS) connectivity strength maps for controls (LD) and individuals who are initiating recovery and will either remain abstinent (ABST) or relapse (REL) across the assessment period at their original assessment (TP1=1 month abstinent). Images on the right reflect the degree of connectivity for the ABST and REL groups at TP2 (~ 3 months later). (Bottom) Images show between-networks connectivity strength maps for the LD group at TP1 as well as for the ABST and REL groups at TP1 (left) and TP2 (right). *Note:* Brighter colors and higher numbers on the color bars indicate regions of interest with relatively greater connectivity losses compared to the LD controls (i.e., less connectivity). *Source:* Mueller and Meyerhoff, 2019.[27] Copyright Society for the Study of Addiction. Reprinted with permission.

Additionally, the research group examined potential genetic modulators of volumetric recovery.[54] In a study of the Val66Met (rs6265) polymorphism in the brain-derived neurotrophic factor gene (BDNF), they found that between weeks 1 and 5 of abstinence, people homozygous for VAL exhibited increases primarily in gray matter volumes, while heterozygous people (VAL/MET genotype) showed volume increases predominately in white matter. However, the total volume was equivalent for both genotypes at each time point (Note that the sample included no individuals homozygous for MET). Neurocognitive improvement was associated with gray matter increases, but not white matter increases. The same polymorphism also was investigated as a modulator of hippocampal change and neurocognitive function across the first 8 months of abstinence in people with AUD who were homozygous for VAL or carried the MET allele (MET hetero- or homozygous).[55] Compared with control subjects without AUD, hippocampal volume was lower in the AUD groups at the initial assessment and remained so across all assessments. However, individuals homozygous for VAL were more likely to show hippocampal volume increases across the test interval. Contrary to other reports from this research group,[44] smoking did not affect initial or recovery measures.

Neurochemical Change in Recovery

Reduction in neurochemical dysregulation has been examined in a relatively small body of work. Zahr and colleagues[56] examined levels of NAA, Cho, CR, and glutamate in recently abstinent individuals with AUD (mean days abstinent = 19.6 ± 12.6) and control participants. NAA and Cho levels were inversely affected by pretreatment drinking variables. Of particular interest were findings showing that reduced levels of NAA in the thalamus were found mainly in individuals who would relapse in the 3 months following treatment.

Prisciandaro and colleagues[57] examined changes in GABA, glutamate, and glutamine by conducting three magnetic resonance spectroscopy sessions across a 1-week monitored abstinence period (i.e., on days 1, 3, and 7) in non–treatment-seeking individuals meeting criteria for an AUD. The participants reported an average of 7.2 drinks/drinking day with an average of 7.8 heavy drinking days (i.e., ≥ 5/4 drinks in a day for men/women, respectively) across the previous 2 weeks. Outcomes showed a significant increase (i.e., normalization) of GABA between scans 1 and 2, without subsequent additional change. In contrast to another report from this research group,[25] changes in glutamate and glutamine were not robust. Age, which ranged from 21 to 40, did not impact outcomes. There were insufficient numbers of women to permit analysis by sex. The investigators concluded that the difference in outcomes across their studies may be related to sample differences in severity of AUD.

Summary

The studies reviewed here offer significant insight regarding brain changes in AUD. Unfortunately, women constituted only a small percentage of the study samples, and thus sex differences cannot be adequately explored. Furthermore, much of the published research cited above derives from the efforts of a single research group, and the samples in the separate reports overlap substantially. Given the realities of human neuroimaging studies (i.e., subject costs, selection criteria, resource availability), sample overlap across investigations to ensure study efficiency is not unexpected. While this pattern does not detract from the potential import of the work, it demonstrates the need to replicate the work and expand the samples to allow for evaluation of sex effects.

INTERVENTION STRATEGIES

An important next question is to what degree the neurobiological and neurobehavioral deficits associated with AUD can be impacted by active interventions. The following sections briefly introduce behavioral and pharmacologic strategies that may facilitate neurobiobehavioral recovery and improve long-term outcomes.[2] Other approaches, including neuromodulation, are gaining

momentum as possible interventions for substance use disorders[58] but will not be discussed.

Cognitive Training/Rehabilitation

Examination of cognitive training in AUD has a long history, but few systematic studies were conducted until relatively recently.[2,30] Performance improvement across training tasks is referred to as "gains," while the impact of training on additional (untrained) tasks constitutes "transfer of training." Adaptive training protocols, which adjust to the skill level of the participant, are more efficacious in facilitating training gains and transfer of training, particularly to novel tasks reliant on the trained process (i.e., proximal transfer), than are nonadjusting training protocols.[59] A key issue is the degree to which training transfers to performance on untrained processes (i.e., distal transfer).

Several examinations applying multi-domain training paradigms reported training-dependent improvements across broad measures. Rupp and colleagues[60] demonstrated improvements in attention and memory performance among treatment-seeking individuals with AUD. Improvements were observed in several cognitive measures, with multivariate analyses suggesting substantial transfer across tasks. Gamito and colleagues[61] administered a web-based training to individuals with AUD during inpatient treatment. Results suggested training-associated improvements in composite scores on a battery of executive function tasks. Fals-Stewart and Lam[62] examined training effects in a 6-month intervention program. Using a training battery engaging diverse neuropsychological domains, they observed transfer to an untrained neuropsychological battery.

In contrast to multi-domain training, contemporary studies often focus on single-domain approaches. Jones and colleagues[63] investigated training with an inhibitory control task. Despite use of a stop-signal paradigm as both a training and outcome measure, they did not note training-associated improvements. Beyond that study, inhibitory control training remains relatively rare among AUD-focused training examinations, despite its relevance to abstinence maintenance. Other single-domain training approaches have assessed memory improvement. Bell and colleagues[64] used a training protocol directed at increasing memory capacity among veterans with AUD. They detected training-associated transfer for untrained verbal memory and learning measures. Most of the recent alcohol-related training investigations have used working memory training. Gunn and colleagues[65] observed proximal transfer on three of six nontrained working memory tasks, two of which continued to display improvement at a 1-month follow-up assessment. Khemiri and colleagues[66] determined transfer in one verbal working memory task, but no improvement across several additional measures, including alternate working memory tasks. Similarly, Hendershot and colleagues[67] identified training-associated improvement in a digit span task, but not in three other working memory transfer measures. Snider and colleagues[68] observed proximal transfer using a "functional" working memory task wherein participants followed a set of sequential object manipulation instructions. In addition to enhanced performance on a functional assessment, this study also noted gains in delay discounting. Although similar assessments of distal transfer remain rare, a recent pilot study suggested that incorporation of emotionally valent stimuli in working memory training may facilitate transfer to social cognition outcomes.[69]

Together, these investigations support assertions that cognitive training may be a useful tool to accelerate cognitive recovery in people with AUD. Proximal transfer has been observed across numerous training studies, while distal transfer has been less commonly examined and, when studied, inconsistently observed. If these interventions are to be effectively utilized, individual and methodological variables contributing to outcome heterogeneity must be systematically interrogated and defined.

Cognitive Enhancing Medication

Despite substantive efforts directed at drug development for AUD,[70] improvement in alcohol-associated cognitive deficits has received little consideration as a primary measure of efficacy. Among the FDA-approved medications for AUD, older studies found little impact of naltrexone, subtle decrements resulting from disulfiram, and some putative benefits associated with acamprosate.[71] A comprehensive review of current AUD-focused drug development efforts is beyond the scope of this article. However, given their demonstrated potential to benefit brain function as evidenced by neurocognitive performance, potential glutamatergic and cholinergic AUD pharmacotherapeutics bear mention.

Glutamatergic medications

NMDA glutamate receptors (NMDARs) are integral to learning/memory function, alcohol cue salience, incentive motivation for alcohol use, and mediation of withdrawal-associated neurotoxicity.[72] Memantine is an FDA-approved, noncompetitive NMDAR channel blocker that may improve AUD-associated outcomes.[73] In preclinical studies, memantine conferred neuroprotection from withdrawal-associated damage[74] and ameliorated withdrawal-associated cognitive deficits.[75] In clinical studies, memantine improved behavioral symptoms and cognitive deficits in alcohol-related dementia.[76] However, a recent double-blind, placebo-controlled pilot study of treatment-seeking individuals with AUD demonstrated no cognitive benefit.[77]

Cholinergic medications

Neuronal nicotinic acetylcholine receptors (nAChRs) are activated by alcohol, facilitating mesolimbic dopamine release.[78] Animal models indicate a substantive role of nAChRs in mediating both alcohol consumption and relapse behaviors. Taken together with the high prevalence of nicotine use in people with AUD, extant data suggest that nAChR agonists may be useful as putative pharmacotherapies for AUD.[79] Varenicline is an nAChR agonist with FDA approval for supporting smoking cessation. Varenicline also reduces alcohol consumption among individuals with AUD.[80] Roberts and McKee[81] recently examined varenicline-associated cognitive alterations in people with AUD. One week of varenicline administration appeared sufficient to induce dose-dependent improvements in working memory performance and reaction time relative to placebo. At the highest varenicline dose, improvement in working memory performance was associated with larger reductions in alcohol consumption. Galantamine, an nAChR agonist and acetylcholinesterase inhibitor,[82] appears to reduce relapse severity.[83] Galantamine appears to improve sustained attention and working memory functions among abstinent individuals with psychostimulant use disorders;[84] however, its cognitive effects in people with AUD have not been investigated.

Summary

It is possible that alcohol-related cognitive deficits can be mitigated by behavioral, pharmacologic, or combination therapies. The current body of research is insufficient to draw strong conclusions. Yet, evolving data indicate the promise of systematic research regarding a range of treatment alternatives, both separately and in combination. A critical part of this research must address the fact that extant data cannot fully answer the related question whether these interventions, if successful in improving cognition, impact long-term alcohol use patterns. Thus, the path forward requires a highly programmatic approach.

CONCLUSIONS, LIMITATIONS, AND FUTURE DIRECTIONS

A large body of research has examined the persistence of alcohol-related neurobiological and behavioral compromise after detoxification. Encouraging data, acquired across decades of research, have revealed a reduction in impairment following the initiation of abstinence. Significant neurobehavioral improvement has been observed in the early weeks of abstinence, with some continuing recovery in later months. For some

measures, deficits are mitigated, but measurable compromise persists compared with healthy controls. Similar conclusions can be drawn regarding improvement in neurophysiological measures, brain volume, neurochemistry, white matter integrity, and brain network integration/activation. One of the most striking outcomes is the substantial research suggesting that improvement is contingent on sustained abstinence. Increased age frequently is associated with less effective recovery. Limited data are available regarding sex differences, with inconsistent results, and still fewer studies have considered the interaction of age and sex. Finally, it is important to keep in mind that adaptive behavior change may occur even in the absence of substantial structural or neurophysiological "recovery" compared with initial brain or behavior compromise. These adaptations may be mediated by the engagement of compensatory mechanisms/processes, such as sacrificing response speed to enhance accuracy or engaging alternate or additional brain areas. This issue remains largely understudied in the context of AUD recovery.[4]

One strength of current research is the ability to probe the interrelationships of structure and function. As shown in previous sections, developing science extends and clarifies earlier conclusions and affords the opportunity to disentangle neurobiobehavioral processes that may differentially contribute to improvement. These advances promote both scientific and clinical progress. For example, Galandra and colleagues[23] demonstrated that alcohol-related deficits in aspects of executive functions may be mediated by dysregulation in the salience network. Based on current understanding of the functions and underlying structure of the salience network, this finding is consistent with cognitive frameworks that emphasize failures in active ignoring as a core component of alcohol-related executive function deficits. Together, the neurobiological and behavioral data provide a rationale for the testable hypothesis that improving the ability to ignore irrelevant stimuli (i.e., enhancing active ignoring skills) may be a useful target for behavioral interventions. Similarly, existing research suggests that programmatic integration of cognitive training interventions and cognitive enhancing medications, as well as evolving technologies such as neuromodulation, may accelerate cognitive recovery and ultimately long-term outcomes.

Despite the promise of existing data, there are notable limitations. First, although there are notable exceptions, post-treatment outcomes are often ascertained across a few weeks or months. Thus, long-term trajectories remain understudied. Second, the complexity of conducting systematic longitudinal studies is daunting. Thus, investigators must take full advantage of available data, resources, and volunteers. The result is that a limited sample may contribute to numerous, interdependent studies. Consequently, the findings from a body of work where the supporting studies are populated by overlapping samples may not be generalizable. Third, as noted in the introduction, individual differences are understudied. To the extent possible, this review has discussed the influence of age and sex. However, other less immediately obvious individual variables, such as nutritional status, also are pertinent,[85] but were beyond the scope of this review. Finally, as summarized above, sustained abstinence was required to show improvement across many of these studies. Moreover, participants in the large majority of these studies were individuals seeking treatment, often in inpatient or intensive outpatient facilities and typically meeting criteria for more severe AUD. Thus, the findings described here do not address outcomes among individuals who meet criteria for AUD but who engage in non–abstinence-based treatment or initiate recovery without employing formal treatment programs. A person's selected pathway to recovery is, no doubt, influenced by significant environmental and individual variables that may, themselves, be associated with differential baseline compromise and recovery trajectories. Therefore, all efforts to advance science and practice must take into consideration alternative definitions of "recovery."[86]

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Sex Differences in the Neurobiology of Alcohol Use Disorder

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Sex differences may play a critical role in modulating how chronic or heavy alcohol use impacts the brain to cause the development of alcohol use disorder (AUD). AUD is a multifaceted and complex disorder driven by changes in key neurobiological structures that regulate executive function, memory, and stress. A three-stage framework of addiction (binge/intoxication; withdrawal/negative affect; preoccupation/anticipation) has been useful for conceptualizing the complexities of AUD and other addictions. Initially, alcohol drinking causes short-term effects that involve signaling mediated by several neurotransmitter systems such as dopamine, corticotropin releasing factor, and glutamate. With continued intoxication, alcohol leads to dysfunctional behaviors that are thought to be due in part to alterations of these and other neurotransmitter systems, along with alterations in neural pathways connecting prefrontal and limbic structures. Using the three-stage framework, this review highlights examples of research examining sex differences in drinking and differential modulation of neural systems contributing to the development of AUD. New insights addressing the role of sex differences in AUD are advancing the field forward by uncovering the complex interactions that mediate vulnerability.

KEY WORDS: alcohol use disorder; animal models; sex differences; stress; adolescence; alcohol; brain

BACKGROUND

Addiction is a chronic relapsing disorder characterized by continued substance misuse despite harmful consequences. Alcohol use disorder (AUD) is specific to the maladaptive consumption of alcohol.[1,2] The fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5), published by the American Psychiatric Association, describes AUD by mild, moderate, and severe subclassifications depending on the number of criteria met for the diagnosis.[3] These criteria

include symptoms of (1) compulsive excessive drinking; (2) persistent desire to consume alcohol and unsuccessful efforts to quit; (3) increased time spent in activities necessary to obtain, consume, and recover from alcohol; (4) craving or strong desire to consume alcohol; (5) recurrent use of alcohol that disrupts obligations such as work, school, or home; (6) continued use of alcohol despite persistent social or interpersonal problems; (7) important social, recreational, or occupational activities are reduced; (8) drinking persists in situations that cause harm to the individual or others; (9) consumption persists despite knowledge of the detrimental effects caused by alcohol; (10) tolerance for alcohol by having a diminished effect with the same amount or needing increased amounts for the same effect; and (11) symptoms of alcohol withdrawal. Mild AUD meets two or three of the criteria, moderate AUD meets four or five of the criteria, and severe AUD meets six or more of the 11 total criteria. The severity diagnosis for AUD could be useful for determining distinct neurobiological profiles that may be associated with mild, moderate, and severe AUD. Importantly, preclinical and clinical studies that include sex as a biological factor in experimental design will be essential to fully understand these complex neurobiological mechanisms.

OVERVIEW

The goal of this review is to discuss AUD using the three-stage framework of addiction—binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation[4]—to highlight examples of sex differences in drinking and related behaviors and to describe some of the neurobiological systems underlying AUD. There has been a recent upsurge in clinical studies in humans and experimental studies in animals in which females are included in the experimental design to elucidate the role of sex in the transition from alcohol use, to alcohol misuse, and ultimately to AUD. Sex differences may influence the three phases of addiction and consequently impact AUD risk differently in men and women.[5] The approach of considering sex as a biological factor in study design has gained even more traction because the gap between men and women in the prevalence of AUD has been closing in the past few years.[6,7]

This review focuses primarily on preclinical animal studies using self-administration procedures to elicit alcohol exposure and/or to measure drinking behaviors to allow for more direct comparison to key findings about drinking behaviors in humans. Preclinical drinking models are summarized in other reviews.[8-12] This article also considers the implications of sex on the onset of drinking, the exacerbation of the negative consequences of drinking, and the increased cue-induced relapse in more advanced stages of AUD. Overall, by presenting examples of studies that address sex differences within these stages, this review aims to show the dynamic role sex differences may have on vulnerability to the development of AUD, to generate enthusiasm for studying sex differences in preclinical and clinical alcohol research, and to advance our understanding and treatment of AUD.

BINGE/INTOXICATION STAGE

In this phase, individuals consume enough alcohol to induce intoxication and cause impairment of physical and mental abilities. An example of this is binge drinking—the excessive consumption of alcohol that results in blood alcohol levels of 0.08 gram percent (g/dL) or higher—typically reached by consumption of five or more drinks in men and four or more drinks in women within a 2-hour period.[12-15] When individuals first start binge drinking, they may not experience any physiological or emotional changes of withdrawal when the alcohol wears off; however, this changes over time.

AUD Prevalence and Age at Drinking Onset

The lifetime prevalence of AUD is 29% in the United States, with a higher prevalence in men than women.[2] In the United States, 33% of men and 17% of women binge drink at least once a month, and longitudinal studies suggest that this gap is narrowing due to a decline in frequency

among men.[15] Sex differences in AUD prevalence may relate to the age at drinking onset or an individual's first experiences with drinking alcohol—especially if alcohol consumption is high enough to elicit intoxication.[16,17] The lifetime risk of AUD quadruples when drinking begins on or before age 14 versus age 18,[18] and the factors motivating individuals to first start drinking and to drink heavily differ with sex.[16,17]

Higher risk-taking tendencies can lead to early-onset use and subsequent alcohol misuse—especially in males.[17] Adolescent boys reported "risk taking" and "curiosity" as motivators for drinking alcohol, whereas this was not the case in adolescent girls.[17] Adolescent boys also have higher levels of impulsivity and sensation seeking compared to adolescent girls.[19] Likewise, men have lower aversion to risk in a social context compared to women, which may lead men to engage in more risk-taking behaviors.[20] Interestingly, a significant positive relationship between sensation seeking and alcohol-related risks such as driving under the influence has been observed in women, but not men.[19] This suggests that women with high sensation-seeking tendencies may have an increased chance of causing harm to themselves and others after drinking alcohol compared to men with the same sensation-seeking tendencies. Alcohol-induced increases in risk-taking behavior also have been shown to differ by sex in rodents, with adolescent male rats engaging in higher risk-taking behavior after drinking alcohol compared to adolescent female rats.[21]

Another reason that individuals may drink alcohol is for its acute anxiolytic, or anxiety-reducing, properties. Experimenter-administered alcohol intoxication can temporarily reduce anxiety-like behavior in rodents.[22] Adolescent girls are more likely than adolescent boys to report drinking alcohol to alleviate stress, social isolation, and psychological distress.[23] Similarly, female mice are more sensitive to the anxiolytic effects of experimenter-administered alcohol compared to males, indexed by increased time spent in the open arms of an elevated plus maze.[24] Notably, the anxiety-reducing properties of alcohol are short-lived, experienced only during and immediately following alcohol drinking. As discussed later, and previously reviewed,[25] there is a rebounding effect during the withdrawal phase after alcohol wears off, and the degree of negative affect and altered stress hormone levels experienced at that time differs with sex.

Overall, these studies suggest that sex plays a distinct role in the motivating factors leading to drinking initiation. Risk-taking behaviors are more likely to influence adolescent boys to consume alcohol, whereas adolescent girls are more likely to consume alcohol due to its anxiety-reducing properties. Understanding the factors underlying early alcohol drinking onset may produce better strategies to prevent and dissuade alcohol consumption in adolescence and may help create specialized alternatives to alleviate the need for this coping mechanism.

Frontal Lobe Development and Early-Onset Drinking

Drinking during adolescence has been shown to lead to higher levels of drinking in adulthood in both male and female mice.[26] Heightened levels of risky behavior, such as binge drinking, during adolescence is thought to occur, at least in part, because the frontal lobes are still undergoing significant development during this time. Through its connections to other cortical regions and subcortical limbic structures, the prefrontal cortex coordinates higher executive function and behavior including decision making, stress responses, working memory, and attention.[9,27-29] The anterior cingulate cortex is one of the medial prefrontal regions that is negatively impacted by alcohol drinking, with more pronounced effects in adolescent male rodents and young men compared to adolescent female rodents and young women.[30-32]

Imaging studies in humans show other prefrontal regions are also altered with alcohol drinking in adolescence and early adulthood. The dorsolateral prefrontal cortex is thinner in younger adults who frequently engage in heavy drinking (≥5 drinks) compared to controls, and the magnitude of this effect is more robust in young

adult men compared to young adult women.[32] Binge drinking is associated with lower cortical volume and thickness in adolescent boys versus higher cortical volume and thickness in adolescent girls.[33-35] Notably, alcohol-naïve adolescent boys and girls with a family history of AUD have thinner orbitofrontal cortices compared to age-matched adolescents without a family history of AUD, indicating that some cortical differences precede alcohol misuse.[36] Considering these findings altogether, it is conceivable that an underdeveloped prefrontal cortex may promote early-onset of alcohol drinking, which could further delay or perturb this development—especially in boys and young men—and increase their lifetime risk of developing AUD.

Gonadal Hormones and Dopamine

Reward comprises learning (cue associations), hedonic ("liking"), and motivational ("wanting") components.[37] Conditioned stimuli are initially associated with a reward, but can become motivational cues on their own, incentivizing both appetitive approach and consummatory behavior.[37,38] Female rats show more appetitive approach, measured by the total number of head entries into a dipper access area (dipper approaches) and have higher levels of lever presses (active lever approaches) to obtain the alcohol reward.[39] Consummatory behavior, measured by the number of dipper presentations into the access area (reinforcers delivered) is also higher in female rats compared to male rats.[39] This is consistent with other rodent studies showing that females consume more alcohol relative to body weight and engage in higher levels of cue-mediated alcohol-seeking behaviors compared to males.[40-42]

The mesocorticolimbic dopamine pathway may contribute to sex differences in appetitive and consummatory behaviors, given its essential role in conditioning and associative learning of environmental and physiological cues that predict alcohol reward availability.[39,43-45] Alcohol binge drinking activates cells in the ventral tegmental area (VTA) of the mesocorticolimbic dopamine pathway.[45-47] This midbrain structure is the origin of dopaminergic cells that project to the ventral striatum (nucleus accumbens), frontal cortex, and amygdala. Rats will press a lever to self-administer alcohol directly into the VTA, but a higher dose of alcohol is needed for reinforcement of this behavior in males compared to females.[48,49] Moreover, a prior history of adolescent intermittent alcohol exposure leads to heightened sensitivity to the rewarding properties of alcohol in both sexes, indexed by a leftward shift in alcohol dose-response curves in rats.[48] In humans, a familial history of AUD is associated with an exaggerated ventral striatum dopamine response to the expectation of alcohol.[50] Although this study did not find a sex difference in this dopamine response, perhaps a larger number of subjects would be needed to detect a subtle, but statistically significant, difference in this measure in men and women.[50] Nevertheless, it is important to consider how dopamine contributes to sex differences in AUD vulnerability, given the role dopaminergic cells in the VTA play in reinforcement learning and in expectation of alcohol availability.

The interaction between gonadal hormones and dopamine may provide insight into the molecular mechanisms underlying sex differences in the rewarding properties of alcohol.[51,52] Estradiol enhances the stimulating effect of alcohol on VTA dopamine neurons.[51] In vitro extracellular recordings of dopaminergic neurons have been conducted using VTA slices obtained from female mice under the following hormonal conditions: no estradiol (ovariectomized and vehicle-treated) or low circulating levels of estradiol (gonadally intact mice in estrus) versus moderate (gonadally intact mice in diestrus II) or high (ovariectomized mice treated with proestrus-like levels of estradiol benzoate) circulating levels of estradiol.[51] Alcohol increased excitation of VTA dopamine neurons in brain slices from mice of all hormonal conditions, but the effects were most robust when estradiol levels were moderate or high.

Lastly, in vitro treatment with ICI 182,780—an antagonist of estrogen receptor subtypes alpha and beta (ERα and ERβ, respectively)—attenuated alcohol-induced excitation of VTA dopamine

neurons in mice with moderate levels of estradiol (diestrus II); this suggests that estradiol's modulation of dopamine sensitivity to alcohol may be occurring through its acute interaction with ERα and/or ERβ subtype in the VTA slice. The acute interaction between estradiol and its receptors appears to depend on moderate or high estradiol levels, as the ERα/ERβ antagonist did not measurably attenuate alcohol-induced increases in dopamine firing under conditions of low estradiol (estrus).

Through its effects on mesocorticolimbic dopamine, estradiol appears to mediate association-based learning and the rewarding properties of alcohol in context, which could ultimately promote drinking. Indeed, estradiol-treated ovariectomized mice show both increased dopamine signaling in the VTA in response to alcohol and increased preference of an alcohol context compared to vehicle-treated ovariectomized mice.[53] The preference for an alcohol-paired context suggests that estradiol enhances the rewarding effects of alcohol.[53] Estradiol also increases alcohol consumption in these mice and inhibition of either ERα or ERβ blocks this effect, suggesting that co-activation of both receptor subtypes is dependent on estradiol.[53]

Progesterone and its metabolites also have been implicated in the modulation of mesocorticolimbic dopamine neurons in response to alcohol.[54] A study in male rats showed that progesterone increases the dopamine extracellular concentration in the medial prefrontal cortex after an experimenter delivered administration of alcohol, inducing a 55% increase compared to controls.[54] Alcohol intake also increases brain concentrations of allopregnanolone (3a-hydroxy-5a-pregnan-20-one)—a neuroactive metabolite of progesterone.[55] Nonhuman primate research in females shows that drinking levels increase when serum levels of estradiol and progesterone and its metabolites are higher (i.e., during the luteal phase compared to the follicular phase of the menstrual cycle).[56] Within the luteal phase the highest drinking occurred on the declining phase of the progesterone peak, with a trend of a positive correlation between serum allopregnanolone levels and alcohol intake.[56] Progesterone and neuroactive steroids could be modifying drinking behavior through effects on mesocorticolimbic dopaminergic neurons involved in reward processing, but more research is needed to understand sex differences in these effects.[54]

Sensitivity to the Aversive Consequences of Drinking

Binge drinking can cause injuries and other adverse outcomes, with high-intensity (extreme binge) drinking (10 or more drinks in men, eight or more drinks in women) resulting in more severe consequences such as blackouts, alcohol overdose, and even death.[57] Some of the short-term aversive consequences of alcohol intoxication can help curtail continued alcohol consumption; yet, these are more subdued during adolescence, and in males in particular.[57] Adolescent boys are less prone to the negative effects of alcohol after a binge-drinking episode, taking less time to recover from alcohol intoxication compared to adolescent girls.[23] Similar trends of decreased sensitivity to the aversive properties of alcohol have been reported in male rodents, but this varies with age, species, and other factors.[58-61] Nevertheless, reduced sensitivity to the aversive properties of alcohol may contribute to higher levels of binge and extreme binge drinking in adolescent boys compared to adolescent girls, which ultimately could lead to differential risk of AUD in adulthood.[57]

WITHDRAWAL/NEGATIVE AFFECT STAGE

After repeated episodes of binge drinking, individuals can begin to experience a negative affective state when alcohol is withdrawn voluntarily or involuntarily. This includes dysregulated stress hormone levels, dysphoria, anxiety, depression, and irritability—a symptomology thought to be due in part to adaptations in stress-related neural pathways.[9,62,63] Experiencing these aversive symptoms when alcohol wears off can set up a strong cyclical pattern of negative reinforcement in

which individuals learn that if they consume alcohol again, they can "feel normal"—at least temporarily.

Negative Affective State During Alcohol Withdrawal

Chronic heavy alcohol consumption eventually can lead to severe AUD. A hallmark feature of AUD is the negative emotional and physiological state that arises when alcohol wears off.[64] Individuals may experience a combination of various symptoms ranging from dizziness to headaches, irritability, anxiety, dysphoria, sleep disturbances, and hypersensitivity to pain.[3] As mentioned above, it has been proposed that alcohol dependence arises because individuals go through repeated cycles in which alcohol consumption serves to mediate the effects of withdrawal, acting as a negative reinforcer.[5,25,45,65,66] A negative reinforcer is a driving force that—with the removal of an aversive stimulus such as negative affective state during withdrawal—promotes a specific behavioral response such as drinking relapse.[65]

Individuals with AUD report having negative and unpleasant feelings during withdrawal, such as low self-concept, neuroticism, depression, and hostility—all of which predict alcohol craving.[67,68] Behavioral assays also have been developed to assess a negative affective state experienced during withdrawal in animals. In addition to the traditional assays such as the elevated plus maze and open field, the frequency of ultrasonic vocalizations also can be measured to assess anxiety-like symptoms of negative affect that are experienced early after withdrawal from chronic alcohol exposure in rodents.[69,70] A recent study used this measure to examine sex differences in withdrawal-induced negative affect in rats that were exposed to 6 weeks of intermittent alcohol.[71] The researchers found that male rats increased the frequency of vocalizations during acute withdrawal, whereas female rats did not.[71] A difference in withdrawal sensitivity may incentivize continued heavy alcohol use to a greater degree in males compared to females, thus putting them at a higher risk of AUD.

Male rats and mice show a more pronounced display of negative affective-like behaviors and neuroactivity after withdrawal from chronic alcohol exposure compared to female rats and mice.[71-75] Alterations in glutamate signaling from the stria terminalis projecting into the basolateral amygdala are thought to mediate these behavioral differences.[73,76] Shorter duration of exposure to chronic intermittent alcohol vapor intoxication and withdrawal cycles was sufficient to detect these synaptic alterations in male rats versus female rats.[73] Furthermore, a translational study using magnetic resonance spectroscopy showed that rats exposed to chronic intermittent alcohol vapors and people diagnosed with AUD have increased glutamatergic neurotransmission during acute alcohol withdrawal compared to their respective controls.[77]

Dysregulation of Stress Hormones

Withdrawal from alcohol is associated with a dysregulation of stress hormones. The hypothalamic pituitary adrenal (HPA) axis governs the neuroendocrine response to stress by releasing corticotropin-releasing factor (CRF) from the hypothalamus, which activates the release of the adrenocorticotropic hormone (ACTH) from the anterior pituitary, resulting in the release of the glucocorticoids from the adrenal glands (cortisol in primates and corticosterone in rodents).

Studies in humans show that, compared to men, women had lower ACTH and cortisol levels under baseline (resting) conditions in the morning, but were more sensitive to peripheral stimulation of the HPA axis as indexed by the dexamethasone/CRF test.[78] In contrast, men showed a greater response than women to the centrally acting citalopram stimulation test.[78] This test measures the extent to which a selective serotonin-reuptake inhibitor acts specifically on the hypothalamus to initiate a stress response. Compared to women, men also exhibited greater activation in response to stress of corticolimbic structures including the medial prefrontal cortex, the extended amygdala and posterior insula, and the hippocampus.[79] In rodents, HPA activity is higher in females under basal (stress-free) conditions and in response

to an acute stress challenge.[25,80-82] In rodents, stress experienced in utero can exaggerate these sex differences even more by enhancing HPA responses in females and dampening it in males.[83]

In male rats, dampened HPA responsivity has been observed after withdrawal from chronic intermittent alcohol vapor exposure, and to a lesser extent following chronic alcohol drinking alone.[84] Although sex differences in corticosterone responsivity were not directly tested, corticosterone responsivity appears to differ 24 hours into withdrawal from chronic alcohol drinking and following predator order stress in male and female mice.[81] Studies in nonhuman primates and rodents have confirmed that alcohol drinking acutely elevates blood levels of ACTH and glucocorticoids.[81,84-86] It is thought that repeated cycles of intoxication and withdrawal eventually desensitize this system, resulting in neuroendocrine tolerance to alcohol.[9,87]

Dysregulation of the HPA axis is thought to result from alcohol-induced neuroadaptive changes within this neuroendocrine axis itself.[84] Glucocorticoid receptor signaling is required for the development of dependence, but it remains unknown whether the accompanying neuroendocrine tolerance contributes functionally to escalated drinking after dependence.[9,88] In addition to the HPA axis, there are neuroadaptive changes in other stress regulatory pathways as well such as the prefrontal cortex, bed nucleus of the stria terminalis, and central amygdala.[9,47,88-91]

Stress can increase alcohol drinking, but this depends on sex, age, and the type of stress exposure.[81,92] Adult female rodents show higher drinking compared to adult males, relative to body weight, and predator odor stress has been shown to elevate drinking in male rodents to the level of drinking observed in females.[40,80] In one study, adult mice had 3 weeks of intermittent binge drinking using the scheduled high alcohol consumption (SHAC) procedure, followed by 1 month of abstinence, and then were tested for alcohol drinking before and following 2 weeks of intermittent predator odor stress (dirty bedding from rats).[81] Among male mice with a prior history of binge drinking, 2 weeks of stress elicited the greatest increase in drinking relative to baseline. This stress effect was found in female mice only when the baseline drinking was stratified into two subgroups: low versus high levels of drinking. Only females that had originally exhibited low drinking levels showed the increase in drinking in response to stress.[81] Female mice that initially exhibited high drinking did not show a further elevation, possibly due to a ceiling effect.

Another study of mice used the "Drinking in the Dark" (DID) binge drinking procedure for 2 weeks followed by 11 days of unpredictable, chronic, mild stress.[93] Afterwards, alcohol drinking was measured with a two-bottle choice of 20% versus 40% v/v alcohol test. Stress increased alcohol binge drinking in both sexes, but this effect was exacerbated even more in male mice with a previous history of drinking prior to stress.[93]

The studies discussed above and others[94] suggest that males may be more susceptible to alcohol withdrawal; however, early-onset drinking can interact with these factors and drive up vulnerability in females. Five days of exposure to restraint stress increased alcohol drinking in adolescent female rats, but decreased drinking in adolescent male and adult female rats.[92] This suggests a heightened sensitivity to stress in adolescence that may have a particularly detrimental impact in females. In support of this, adolescent-onset binge drinking increased anxiety-like behavior early in withdrawal in female mice, and this persisted into abstinence.[95] Likewise, acute stress elicited a negative affective state in the novelty-induced suppression of feeding task in adult female mice with a history of adolescent alcohol exposure.[76] A history of adolescent binge drinking and intermittent alcohol vapor exposure led to a negative affective-like state in the elevated plus maze task and fear conditioning response in male mice, but it did not emerge until later in abstinence.[96]

The neural systems implicated in the interactive effects of stress and alcohol include not only structures of extended amygdala, but also brain regions thought to be involved in the third stage of AUD (preoccupation/anticipation).[73,86,97-100] For

example, a history of prior binge drinking and exposure to predator odor stress dysregulates protein levels of stress-related receptors, and does so in a sex-specific manner.[81] After chronic drinking, there is a measurable increase in glucocorticoid receptors in the prefrontal cortex and hippocampus, and CRF receptor 1 in the hippocampus of female mice, but not male mice.[81] These neuroadaptive changes in stress-regulatory circuits could persist well beyond withdrawal and underlie some of the psychological components that predict craving and relapse.[67]

PREOCCUPATION/ ANTICIPATION STAGE

Prolonged heavy alcohol use leads to a state of a constant preoccupation with alcohol and compulsive drinking despite negative consequences.[88,101,102] This craving can continue into abstinence for months or years, making it difficult to abstain from alcohol altogether or to shift to a healthier level of drinking.[103]

Sensitivity to Alcohol-Related Cues

After long bouts of abstinence, alcohol-related cues can trigger incentive salience, which heightens cravings and precipitates relapse.[37,104,105] Men in particular exhibit higher levels of alcohol craving than do women,[106] and cravings are associated with increased activity in the striatum in men, but not in women.[79] Cue-induced reinstatement procedures are useful for studying the underlying neurobiological mechanisms by which alcohol-related cues promote craving and relapse during abstinence.[107] Like humans, male rodents appear more susceptible to relapse than females.[108] Brain-derived neurotrophic factor (BDNF) may play a role in mediating this sex difference.

In mice, male offspring of alcohol-exposed fathers have high *Bdnf* gene expression in the VTA and low alcohol drinking behavior; this effect was not observed in female offspring.[109] Conversely, genetic manipulation to reduce BDNF protein levels to 50% in female rats resulted in a heightened, male-like, response to alcohol cues.[108] This genetic manipulation had no effect in males. Others have found a sex difference in tropomyosin receptor kinase B (TrkB) signaling in *Bdnf* +/– mice, with males showing higher TrkB phosphorylation than females in the prefrontal cortex and striatum.[110] Consequently, BDNF signaling is presumed to mediate cravings in response to alcohol cues and this increased sensitivity to alcohol-related cues could put males at higher risk of relapse even after long periods of abstinence.

Compulsive Alcohol Drinking After Chronic Use

As discussed earlier, multiple cycles of binge intoxication followed by withdrawal can transition individuals from light to moderate drinking to severe AUD.[5,25,45,66] At this point, heavy drinking can become more compulsive.[111] Compulsive alcohol use is inflexible and persists despite negative consequences or despite devaluation of the rewarding effects of alcohol. This type of drinking is characteristic of physical and motivational/emotional dependence on alcohol.[88,112]

One strategy used to measure inflexible drinking is the assessment of a persistent motivation to drink despite increasing the response requirement to obtain alcohol. In animal studies, this can be tested by training subjects to press a lever or nose poke for alcohol in operant boxes.[9] The number of responses to get the reward can be changed using fixed ratio or progressive ratio schedules of reinforcement in operant alcohol self-administration studies. Fixed ratio is the number of presses necessary for reward delivery, increasing the response requirement for the reward. This challenge measures compulsive-like behavior that is characteristic of addiction, in which individuals go to extreme lengths to obtain the drug on which they are dependent. Progressive ratio takes this a step further and increases the response requirement for reward delivery. In humans, a progressive ratio trial of intravenous alcohol self-administration showed that women increased their work effort to obtain alcohol after resumption following 2 weeks of abstinence, whereas men decreased this effort.[113] Male rats exposed to alcohol vapors to produce

dependence display increased compulsive-like behavior and increased intake on both fixed and progressive ratio schedules.[88] However, progressive ratio tests in Long Evans rats suggest there is no sex difference in motivation for alcohol, at least following extinction and reinstatement of alcohol self-administration.[114] Comprehensive studies are needed to assess compulsive drinking behaviors and relapse after prolonged abstinence in both nondependent and dependent animals to better understand sex differences in AUD.

Alcohol solutions also can be manipulated to devalue reward and to test for signs of inflexible drinking. One approach to devaluing alcohol is the addition of an unpleasant substance to change the flavor of alcohol by adding the bitter taste of quinine hydrochloride dihydrate or lithium chloride.[111] Female mice have been shown to be more resistant to devaluation by quinine than males, and this sex difference was not attributable to differences in sensitivity to quinine.[115] Nevertheless, sex differences in sensitivity to alcohol reward devaluation may be temperament- or species-specific, as male and female Long Evans rats reduce drinking levels to the same extent following alcohol devaluation.[114,116] In addition to alcohol adulteration, more sophisticated procedures derived from behavioral economics can be used to manipulate the value of the reward by changing the alcohol reinforcer magnitude, availability of alternative reinforcers, and delay discounting.[117,118]

Another approach used to test for inflexible drinking is to measure shock-resistant alcohol intake.[112,119] Rodent and human studies use these procedures to measure compulsive alcohol drinking despite negative consequences (e.g., foot shock or electric shock to the wrist, respectively). In rats, when one of eight alcohol-seeking responses are paired with foot shock, half of the alcohol-dependent male rats exhibit shock-resistant alcohol intake.[120] Male alcohol-preferring rats that received an intermittent foot shock in response to alcohol seeking separated behaviorally into three distinct subgroups: (1) compulsive rats that continued alcohol seeking despite punishment, (2) noncompulsive rats that diminished their alcohol-seeking responses, and (3) an intermediate group that only partially suppressed their alcohol-seeking behavior.[119] These two studies did not elucidate a sex difference as neither included female rats in the study design.[119,120] Heavy alcohol use in men and women is associated with risky and inflexible drinking, with men and women with AUD making more attempts to obtain aversion-paired rewards compared to individuals without AUD.[121,122] Furthermore, higher connectivity between the anterior insula and the nucleus accumbens is associated with increased compulsive-like behavior.[122]

Altogether, these studies suggest that inflexible drinking promotes heavy and continued use of alcohol and, consequently, may lead to further neuroadaptations in the brain. However, some of the devaluation strategies show limited evidence of sex differences. The inclusion of female subjects in these studies to directly compare the effects is vital to evaluate the role of sex in compulsive-like drinking under these different paradigms.

Chronic Alcohol Use and Corticolimbic Circuitry

Deficits in executive function can result from early-onset drinking or chronic heavy use, and this may lead to a higher chance of relapse following abstinence.[123] Some of these effects may be due to alterations in connectivity between prefrontal cortices and subcortical structures that are involved in reward processing.[5,124] The medial prefrontal cortex, anterior insula, and striatum are more active and have stronger connections in men and women with AUD compared to controls.[125] This could result in more subcortical control over decision-making processes based on reward reactivity rather than executive control.[125]

With long-term abstinence in both men and women, there is increased resting-state connectivity to brain regions that control executive function and decreased connectivity within reward processing regions.[126] Connectivity between the nucleus accumbens and the orbitofrontal cortex has been observed to be stronger in individuals with a familial history of AUD compared to individuals without this predisposition.[127] These studies suggest that chronic

exposure to alcohol leads to reduced function of the prefrontal cortex, which, when combined with a stronger influence of striatal control over decision-making, can increase the risk of relapse.[125,127]

Animal studies have advanced our understanding of neural connectivity at the axonal and microstructural level, giving insight into the mechanisms by which prefrontal function improves across development and can be impaired after alcohol exposure. During adolescent development in rats, prefrontal axons undergo robust increases in myelin ensheathment, which corresponds with a twofold increase in neuronal transmission speed.[128] Binge drinking during adolescence is also associated with altered neurodevelopmental trajectories including poor frontal white matter integrity in adolescent boys and girls.[129,130]

Longitudinal studies show that white matter growth is attenuated in the frontal lobes in humans who started drinking during adolescence—an effect that was comparable in both sexes.[131,132] The abnormal microstructural development of white matter in the frontostriatal region relates to binge drinking during adolescence and poorer cognitive function.[133,134] Likewise, animal studies show that voluntary alcohol exposure during adolescence decreases the density of myelinated axons in the anterior cingulate subregion of the medial prefrontal cortex, with higher adolescent drinking levels predicting lower working memory performance later in adulthood.[30] Reduced myelin density was not observed in female rats after adolescent binge drinking,[31] which corresponds with another study in mice showing that high doses of alcohol reduce myelin genes to a lesser extent in adolescent females compared to males.[135]

Despite more robust effects in males, examination of myelinated axons at the microstructural level shows that alcohol alters the nodal domain in both male and female rats.[31] The nodes of Ranvier are the ion channel–rich gaps between myelin sheaths on the prefrontal axons, and reduced length-to-width nodal ratios were detected in male and female rats following adolescent binge drinking.[31] In males, the decrease in nodal ratio was due to an increase in nodal diameter after the exposure, whereas in females it was due to a decrease in the nodal length. In both cases, these microstructural alterations have potential to negatively impact the speed and integrity of neural transmission, which is essential for effective communication within and between cortical and subcortical structures.[31] Altogether these studies show alcohol affects cortical circuits that are important for executive functioning and behavioral control, and does so to a greater extent in males than in females.

Administration of extreme binge-like doses of alcohol damages the hippocampus and prefrontal cortex, and impairs memory in rats.[136-138] While damage within the prefrontal cortex was similar in both sexes[138] the severe damage to the dentate gyrus of the hippocampus was greater in females compared to males.[136] The dentate gyrus is a subregion of the hippocampus where new granule neurons are normally produced for the formation of new memories; however, alcohol impairs cell proliferation and reduces the number of granule neurons in this region and does so to a greater extent in females.[136] This damage is associated with a reduction of trophic support molecules and the heightened vulnerability in female rats appears to be due to more robust downregulation of BDNF, insulin-like growth factor 1 (IGF-1), and cyclic adenosine monophosphate (AMP) response element-binding protein (CREB) signaling cascades.[136] These results are consistent with human studies in which the hippocampus was shown to be particularly vulnerable to the effects of alcohol binge drinking.[124,139] Self-administration studies in rodents suggest that even much lower levels of alcohol (low-binge) can decrease neurogenesis and hippocampal size,[140] with reports of alcohol drinking reducing neurogenesis to a greater extent in females compared to males[141] or similarly in both sexes.[142] Hippocampal damage after alcohol drinking in rodents corresponds with significant cognitive and memory dysfunction, especially when the alcohol exposure occurs during adolescence.[26,137,143] Thus, early-onset drinking and chronic heavy alcohol use may eventually lead to sustained hippocampal damage

to a greater extent in female rodents, which in conjunction with prefrontal dysfunction, could interfere with the ability to regulate reactivity to stress and alcohol-related cues that promote craving and relapse.

CONCLUSIONS AND CLINICAL IMPLICATIONS

The preclinical and clinical studies outlined in the current review show sex differences in behavioral risk factors and neural systems implicated in AUD, as summarized in Table 1 and Figure 1. This approach of incorporating sex differences in research studies has enhanced understanding of the complex mechanisms driving alcohol-related behaviors that lead to AUD. An increasing body of evidence shows sex differences in factors contributing to AUD vulnerability during the onset of alcohol drinking and later in the development of severe AUD and relapse following abstinence (see Table 1 for details).

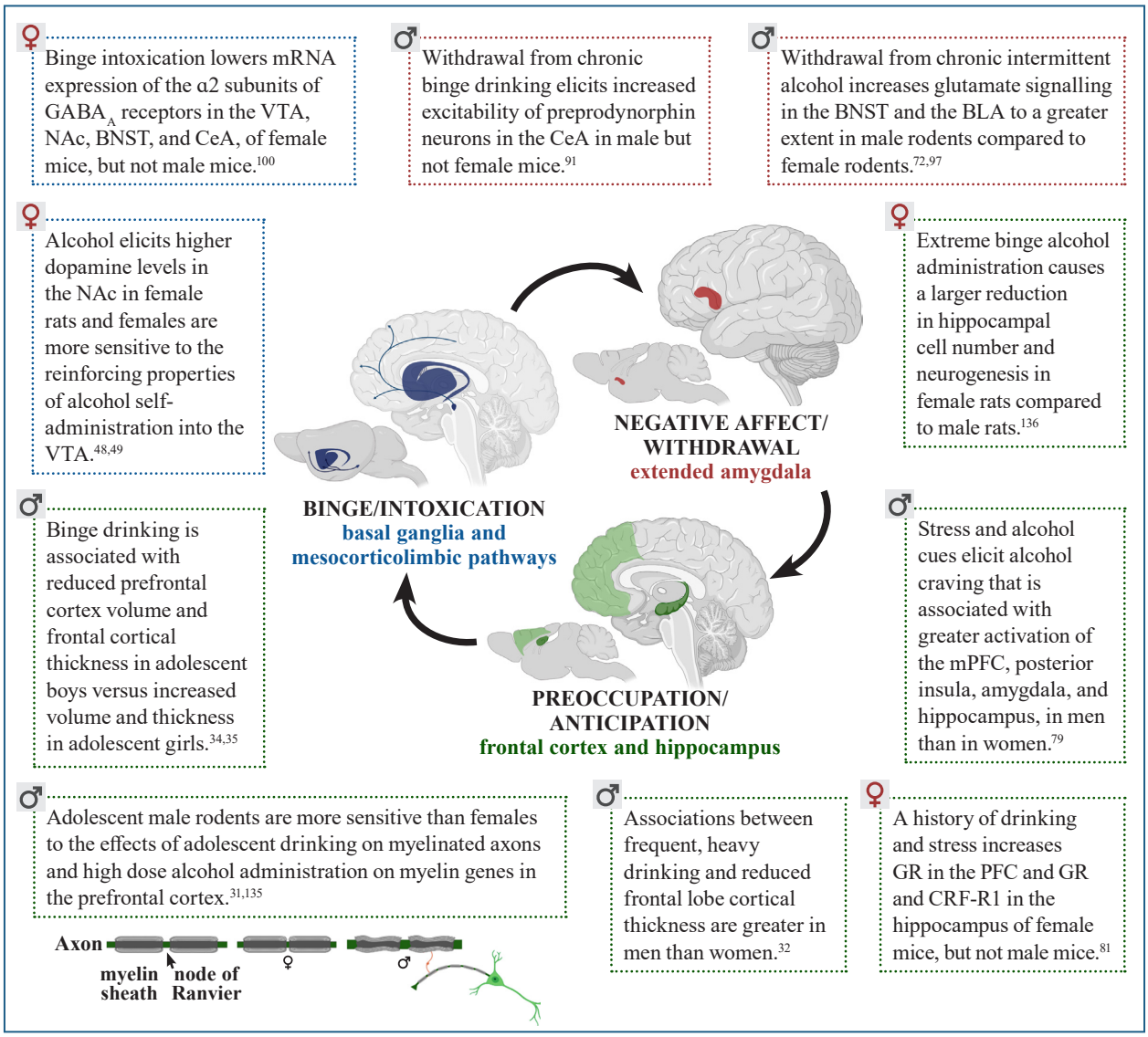

Figure 1 Sex differences in the effects of alcohol on the interacting brain systems associated with the three stages of addiction. *Note:* BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; CRF-R1, corticotropin-releasing factor receptor 1; GABA$_A$ receptors, gamma-aminobutyric acid type A receptors; GR, glucocorticoid receptors; mPFC, medial prefrontal cortex; mRNA, messenger RNA; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area. Created with BioRender.

Table 1 Sex Differences in Behaviors Associated With the Three Stages of Addiction

Binge/Intoxication	
Risk factors that promote early-onset drinking	Impulsivity, a risk factor for adolescent drinking, is higher in adolescent boys compared to girls.[19]
	Drinking to alleviate psychological distress is higher in adolescent girls compared to boys.[23]
Alcohol drinking behavior	Prevalence of binge drinking is higher in adolescent boys compared to girls.[15]
	Appetitive approach in response to a dipper presentation is greater in female rats than male rats.[39]
	Acute alcohol injection increases preference to a large/uncertain reward (a measure of risk-taking behavior) in males, with no preference shown in females.[21]
Withdrawal/Negative Affect	
Alcohol drinking behavior	Restraint stress increases drinking in adolescent female rats, but decreases drinking in adolescent male rats.[92]
	A prior history of adolescent binge drinking augments drinking levels later in adulthood in female mice, but not in male mice.[81]
	Female mice drink more alcohol under baseline conditions in adulthood, but a history of binge drinking and chronic unpredictable stress or predator odor can elevate drinking in male mice to the level of females.[81]
Effects of alcohol withdrawal on negative affect	Adolescent girls report more negative mood states following recent heavy episodic drinking than do adolescent boys.[23]
	A history of adolescent binge drinking elicits active coping responses to stress in female mice vs. passive coping responses to stress in male mice (indexed by less time vs. more time immobile in the forced swim test).[95,96]
	Frequency of ultrasonic vocalizations, a measure of anxiety-like behavior, is increased following withdrawal from chronic intermittent alcohol vapors in male rats, but not females.[69-71]
Preoccupation/Anticipation	
Alcohol drinking behavior	Men exhibit higher levels of alcohol craving in response to cues than women do.[106]
	Women increased work effort in a progressive ratio trial following resumption after 2 weeks of abstinence. Men showed a decrease in effort.[113]
	Relapse-like behavior in response to alcohol availability is higher in male rats compared to female rats.[108]
	Female mice have a higher degree of aversion-resistant drinking than male mice.[115]

Adolescent drinking in the context of stress, negative affect, and increased cue-reactivity is greater in females. Males show vulnerability with regard to higher levels of impulsivity and, compared to females, they are less sensitive to the aversive effects of intoxication, making males less likely to stop drinking. Sex also was found to be a predictor of the negative impact that chronic alcohol use has on the brain (see Figure 1 for details). Males show more severe reductions in cortical thickness and reduced myelinated fiber density in the prefrontal cortex, whereas females show more robust decreases in neurogenesis in the hippocampus in response to alcohol. Sex can specifically influence the effects of alcohol in the brain in the context of intoxication, withdrawal, and cravings, leading to a robust vulnerability to AUD. Overall, these findings show that sex differences in humans and animal models of AUD are also dependent on the unique physiological characteristics of the stages of addiction. Effects of alcohol can be mediated by sex in different directions, by increasing or decreasing vulnerability to AUD depending on

the specific factor being considered. This complex shifting of vulnerability mediated by sex calls for a comprehensive approach toward studying AUD and other addictions.

A number of other health consequences endured after chronic heavy alcohol use are greater in women compared to men. Women with AUD experience higher risks of developing cancers, alcohol-related liver injury, and cardiovascular disease compared to men with AUD despite comparable levels of drinking.[7,25,144-150] Specifically, binge drinking shows an increase of mortality, including cancer-related mortality, and people with AUD have a threefold increase of death and a higher risk of digestive diseases, dementia, cancer, and liver disease. Women with AUD show higher risk of liver disease-related mortality, with 71% of mortality in women compared to 64% in men.[146] Sex differences in the effects of alcohol drinking may be explained in part by the role of gonadal steroid hormones in modulating a variety of functions in the brain. These functions include regulation of hypothalamus-driven social behavior;[151] cognition, memory, and learning driven by the hippocampus and the prefrontal cortex;[152] amygdala-mediated stress responses;[25,153] dopamine-mediated reward;[51] and synaptic plasticity.[154] Moreover, alcohol binge drinking in women can dysregulate the menstrual cycle,[155] which can affect endogenous steroid hormone levels.[156-159]

New diagnostic neuroimaging approaches are being explored to improve the assessment of AUD severity and circumvent limitations of the more traditional methods such as the Alcohol Use Disorders Identification Test (AUDIT) self-report questionnaire. A metabiological study recently reported that resting state connectivity functional magnetic imaging can be useful for assessing AUD.[160] Specifically, differential functional connectivity between the prefrontal cortex and the reward-related areas predicted the severity of AUD with accuracy that surpassed other functional magnetic resonance imaging, structural magnetic resonance imaging, combined magnetic resonance imaging features, or demographic features. The usefulness of these new diagnostic approaches exemplifies the great urgency for more inclusion of female subjects in preclinical AUD studies in humans and animal models. With heightened attention to detail in experimental design and increased consideration of sex/gender differences in interpretation of research findings, we can enhance our understanding of the neurobiological mechanisms underlying AUD to improve diagnosis and treatment in the future.

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Alcohol's Unique Effects on Cognition in Women: A 2020 (Re)view to Envision Future Research and Treatment

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Alcohol use and misuse is increasing among women. Although the prevalence of drinking remains higher in men than women, the gender gap is narrowing. This narrative review focuses on the cognitive sequelae of alcohol consumption in women. Studies of acute alcohol effects on cognition indicate that women typically perform worse than men on tasks requiring divided attention, memory, and decision-making. Beneficial effects of moderate alcohol consumption on cognition have been reported; however, a number of studies have cautioned that other factors may be driving that association. Although chronic heavy drinking affects working memory, visuospatial abilities, balance, emotional processing, and social cognition in women and men, sex differences mark the severity and specific profile of functional deficits. The accelerated or compressed progression of alcohol-related problems and their consequences observed in women relative to men, referred to as "telescoping," highlights sex differences in the pharmacokinetics, pharmacodynamics, cognitive, and psychological consequences of alcohol. Brain volume deficits affecting multiple systems, including frontolimbic and frontocerebellar networks, contribute to impairment. Taken together, sex-related differences highlight the complexity of this chronic disease in women and underscore the relevance of examining the roles of age, drinking patterns, duration of abstinence, medical history, and psychiatric comorbidities in defining and understanding alcohol-related cognitive impairment.

KEY WORDS: alcohol; women; cognition; acute consumption; AUD; recovery

INTRODUCTION

Alcohol use and misuse have increased among women over the past 2 decades,[1] with an estimated 5.3 million women age 18 and older meeting criteria for alcohol use disorder (AUD) in the United States in 2018 (https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-use-disorders). The rate of AUD in women increased 84% over the past decade in comparison with a 35% increase in men.[2] Although the prevalence of men who drink is still higher than that of women, the gender gap is narrowing.[2-4] Of note, prevalence of drinking and binge drinking, defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as four or more alcoholic beverages on the same occasion for women, rose in older women (age 60 and older)[5,6] compared with previously reported levels.

Commensurate with the rising rates of women with AUD should be enhanced efforts to examine sex differences related to consequences of alcohol consumption. Most of the earliest reports of the untoward consequences of alcohol focused on men and suffered from lack of statistical power to identify sex-related differences because of small numbers of female participants or unequal sample sizes between the sexes, raising limits on generalizability to women.[7] Despite this bias, appreciation of sex differences in alcohol-related factors and consequences is not new. Indeed, Lisansky addressed the importance of examining alcohol factors uniquely related to women more than a half century ago.[8] What is new, however, is greater insistence in research studies and clinical applications for systematic investigations to address sex-related differences in alcohol consumption, antecedent factors of drinking, and alcohol-related consequences. As a result of this mandate, work over the past decade has made it amply apparent that men and women differ in alcohol-related risks, health and cognitive consequences, and factors related to successful abstinence and sobriety.[9]

This narrative review focuses on the cognitive sequelae of alcohol use in women, including deficits associated with acute consumption, moderate drinking, at-risk or hazardous drinking, and chronic excessive drinking. (See the box **Effects of Alcohol Consumption on Women and Factors That Influence Research Outcomes**.) Over the years, nomenclature regarding alcohol misuse has changed based on scientific understanding of the disease—for example, "alcohol abuse" and "alcohol dependence" in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) evolved into "alcohol use disorder" by the fifth edition (DSM-5). Although anachronistic for studies predating DSM-5 nomenclature, the term "AUD" is used throughout this review when referring to individuals who met criteria for an alcohol misuse-related diagnosis at the time of assessment.

SEX DIFFERENCES IN ALCOHOL METABOLISM AND THE CONSTRUCT OF "TELESCOPING"

Alcohol is metabolized at different rates in men and women,[10] and these sex differences in the pharmacokinetics of alcohol are biologically founded. Particularly notable is sexual dimorphism of body composition. Compared with men, women generally have less body water and a higher proportion of fat, which does not absorb alcohol, resulting in higher blood alcohol concentration (BAC) levels, even when the amount of alcohol consumed is adjusted for body weight. In addition, women tend to have lower levels of gastric alcohol dehydrogenase, the enzyme that breaks down ethanol into its metabolites. Thus, BAC levels rise faster and stay elevated longer in women than men.[3] It has been speculated that these sex-related pharmacokinetic differences underlie why women can develop health-related consequences, including cirrhosis of the liver, earlier in their disease and after lower total lifetime alcohol consumption than men.[7,11]

"Telescoping" describes the accelerated or compressed progression of the landmark events of AUD (e.g., age at first drink, age when started

Effects of Alcohol Consumption on Women and Factors That Influence Research Outcomes

What We Know

Acute alcohol consumption

Deficits reported in women

- Divided attention
- Psychomotor speed
- Working memory
- Short-term memory
- Set-shifting
- Decision-making

Moderate drinking

Modest beneficial effects

- Better overall cognitive ability
- Slower rate of cognitive decline in aging

Increased risk of

- Breast cancer
- Gastrointestinal disorders
- Infectious diseases

Chronic excessive alcohol consumption

Telescoping

Compared with men:

- Women have shorter intervals between landmark events from the inception of drinking to entering treatment.
- Women experience medical and health-related problems earlier, even when duration and amount of alcohol consumed are comparable between the sexes.
- Women exhibit different patterns and severity of cognitive compromise, some modulated by sex-related emotional and social factors.

Factors That Influence Research Outcomes

* Differences in task demands
* Heterogeneity of response to alcohol
* Small sample sizes
* Differences in study inclusion and exclusion criteria
* Cross-sectional vs. longitudinal study
* Important to control for variables such as
  - Age
  - Education
  - Socioeconomic status (SES)
  - Depression/anxiety symptoms
  - Smoking status
  - Drinking patterns
  - Alcohol-related pharmacokinetics
  - Hormonal differences
  - Nutritional status
  - Comorbid medical conditions
    - HIV
    - Hepatitis C
    - Non-alcohol substance misuse
    - Psychiatric conditions
    - Chronic pain

having problems related to alcohol, age when first entered treatment) in women compared with men.[12,13] Initial studies addressing telescoping focused on duration of time from onset of drinking to time to enter alcohol treatment or time to develop medical problems (e.g., hepatic disease). Early studies reported that women initiate hazardous drinking—drinking that puts a person at heightened risk of developing AUD—at a later age than men, although they enter alcohol treatment earlier in their disease than men.[14,15] Women also were reported to be more susceptible and to experience alcohol-related medical problems after a shorter time of chronic heavy drinking[12] and lower lifetime consumption compared with men.[16] Indeed, there is evidence that women are at heightened risk of alcohol-related heart disease.[3] Taken together, there is increasing support for this phenomenon as it pertains to the physiological and health-related consequences of alcohol in women.[3,17]

Telescoping has been invoked in studies examining the timing and severity of cognitive deficits associated with chronic heavy drinking in women compared with men.[7,18] Demonstration of a shorter duration from drinking to detectable cognitive deficits in women, however, has received mixed support, with some studies supporting the concept of telescoping of select cognitive processes,[18] whereas other studies do not.[19,20] Additional research is needed to examine the temporal sequencing, pattern, and severity of cognitive deficits in women and men in relation to landmark events associated with alcohol consumption. Inconsistency among studies examining the temporal sequence of events related to AUD in men and women could be due in part to methodological or even geographical factors, including accuracy of self-report and factors that mediate and moderate a woman's decision to seek sobriety-related or health-related treatment, such as ease or availability of treatment and help with family responsibilities.[21]

ALCOHOL'S EFFECTS ON COGNITION IN WOMEN

Acute Alcohol Consumption

An early study directly compared the acute effects of alcohol on men and women who were social drinkers without an alcohol misuse diagnosis and reported that, after moderate levels of alcohol consumption (BAC = .04%), women scored lower than men on a short-term memory task.[22] In a study examining divided attention and balance (sway) in light drinkers (12 men—average absolute ethanol intake in the 30 days prior to testing was 7.9 g/kg (range: 5.6-10.0 g/kg), 12 women—7.38 g/kg (range: 5.01-10.23 g/kg); ages 18 to 24), it was reported that the women scored significantly lower on divided attention than the men only at higher alcohol levels (BAC = .06%) and not lower levels (BAC = .03%) or for placebo.[23] Sex-related differences were not observed in sway at any BAC level. Data summarized from seven experiments examining the effects of moderate alcohol dose (0.65 g/kg) in participants with no self-reported history of substance use disorder (ages 21 to 35) on driving performance indicated that these young social drinking women showed greater deficits in memory recall, divided attention, and motor skills than did young social drinking men who did not have AUD.[24] In that review, all driving-related measures were impaired for both men and women after alcohol consumption compared with their nondrinking performance, with women demonstrating a larger decline in performance after drinking than men. These studies provide support for the notion that women may be more vulnerable than men to the cognitive effects of acute intoxication.[16]

By contrast, other studies have failed to find sex differences in relation to acute alcohol consumption. Accordingly, a study assessing 11 men and 13 women found no significant sex differences in performance on cognitive tests including assessment of divided attention, short-term memory, and rotary pursuit at moderate levels of acute consumption, blood alcohol levels (BALs) of .054% for men and .062% for women. BALs were measured at 20-minute intervals after the first drink by using a gas chromatographic intoximeter, and BALs were statistically controlled for in between-group analyses.[25] Additionally, although both men and women were impaired, no sex differences were reported in a study that assessed flight simulation performance in general aviation pilots ages 21 to 40 at moderately high BALs (12 women = .084%, 11 men = .087%), levels exceeding legal limits of intoxication in the United States (BAL = .08%).[26]

Age can moderate the effects of acute alcohol consumption on cognition.[27,28] A double-blind, placebo-controlled factorial design study assessing psychomotor, set-shifting, and working memory processes in community-dwelling social drinkers who had never met criteria for an alcohol misuse diagnosis (15 men, 24 women; ages 55 to 70) at low (breath alcohol concentration [BrAC] = .04%) and moderate (BrAC = .065%) levels of acute alcohol administration reported age-related deficits compared with 51 younger community-dwelling moderate drinkers (31 men, 20 women; ages 25 to 35). Both the younger and older adult groups exhibited some beneficial effect of low-dose alcohol compared with placebo on a simple psychomotor sequencing task (Trail Making Test, Part A). At the higher dose level (BrAC = .065%), however, only the older adults were impaired on a more complex psychomotor task requiring sequencing and working memory (Trail Making Test, Part B).[28] Cognitive efficiency, the ability to perform quickly and accurately, was most compromised in the moderate-dosage group of older adults, regardless of sex.[28]

An examination of acute alcohol effects on cognition failed to identify sex differences in tests of set shifting, psychomotor speed, or working memory in non-problem drinking older adults (26 men, 36 women; ages 55 to 70) randomly assigned to one of three dose conditions: placebo; low dose (BrAC = .040%); and moderate dose (BrAC = .065%).[29] The authors concluded that sub-intoxicating doses of alcohol do not differentially affect healthy, older, moderate-drinking men and women.

Taken together, studies that find sex-related differences on cognitive effects of acute alcohol consumption report that women tended to perform worse than men on higher-order cognitive tasks requiring divided attention, working memory, and decision-making, as opposed to less complex tasks such as reaction time or psychomotor measures.[9] Inconsistency of findings across studies is likely due to a number of factors including subject selection, task demands, and heterogeneity of response to alcohol.

Acute Cognitive Effects of Binge Drinking and Blackouts

Binge drinking can produce blackouts, defined by periods of amnesia (the inability to transfer information from short-term to long-term memory) experienced while an individual is apparently conscious and able to engage in activities such as walking, talking, and driving.[30-32] Rapid increase of BAC is a major risk factor for a blackout, with BAC levels of .22% having upward of a 50% chance of producing a blackout.[33] In young adults, blackouts are a common consequence of binge drinking.[34] Of 2,140 young adults 1 year post high school, 68% reported consuming alcohol at some point in their lifetime, and 20% of that group reported a blackout in the past 6 months.[34] The occurrence of blackouts was as prevalent among young women (17%) as men (22%) in this cohort. Blackouts have been associated with poor decision-making and impulsivity, and they increase the vulnerability of both women and men to unlawful, regrettable, and dangerous interpersonal and social situations. It has been speculated that blackouts could be more predictive than level of consumption of alcohol-related harms.[34]

AUD and Chronic Excessive Consumption

DSM-5 conceptualizes AUD as a chronic relapsing disease, where an individual continues to drink despite knowing that one's current drinking pattern is likely to lead to untoward medical, personal, and social consequences.[35] The diagnosis of AUD is based on a severity continuum ranging from mild to moderate to severe, depending on the number of diagnostic criteria met, which include but are not limited to drinking more than intended, having difficulty refraining from drinking, drinking that interferes with work and family responsibilities, cravings, tolerance, and withdrawal. The AUD continuum differs from the previous diagnostic classification system, DSM-IV-TR,[36] which made a categorical distinction between alcohol abuse and alcohol dependence. Studies investigating the effects of chronic heavy drinking on cognitive processes in women with an alcohol-related diagnosis defined by either DSM system often have reported deficits in line with those in men with an alcohol-related diagnosis, but a number of studies also have reported differences in the cognitive effects of alcohol based on sex, described next.[37-39]

Based on rigorous, quantitative assessments, cognitive deficits associated with chronic heavy drinking in women have been reported since the early 1980s.[19,40] One of the earliest studies compared 33 recently sober women (10 to 23 days since last drink) with 44 age- and education-matched control women on a number of cognitive and motor domains. Impairments were observed in visuospatial processing (block design), psychomotor speed (trail making), information processing (digit symbol substitution), and memory (verbal and visual recognition and recall).[18] The authors of this study noted that the women with AUD displayed significant cognitive and motor deficits, yet had a notably shorter drinking history than participants in previously reported studies that included men with AUD.[18] Indeed, even after statistically controlling for differences in drinking histories between men and women—duration of hazardous drinking in men was more than twice that of women (13 years vs. 6 years, respectively)—and then separately matching men and women on age and years of problem drinking, the study found that women still scored significantly lower than men on tests of memory recall and psychomotor speed.[14] However, it has been cautioned that, given the cross-sectional

nature of the study, it could not be determined whether cognitive deficits in the women were a risk factor for or a consequence of drinking.[14]

The pattern and extent of cognitive and motor deficits across six domains (i.e., executive functions, short-term memory and fluency, declarative memory, visuospatial abilities, upper-limb motor ability, postural stability) were examined in 43 recently sober (average duration, 3.6 months; range 2 to 15 months) women with AUD ages 28 to 63.[41] Compared with 47 no- to low-drinking control women matched on education and scores standardized on age, the women with AUD demonstrated deficits in verbal and nonverbal working memory, visuospatial abilities, and postural stability (balance and gait), with relative sparing of executive functions, declarative memory, and upper limb strength and speed.[41] By comparison, an earlier study examining the pattern and extent of cognitive deficits in 71 recently (1 month) sober men with AUD—compared with 74 healthy control men—reported deficits in executive function, visuospatial abilities, and gait and balance in men with AUD.[42] Taken together, these studies demonstrated that both women and men with AUD showed impairment on visuospatial processes; however, compared with nondrinking, sex-matched control participants, only the women were impaired on tasks of short-term memory, and only the men exhibited executive function deficits.

In a more recent cross-sectional study of 164 older DSM-IV alcohol-dependent participants (62 women, 102 men; age 62.6 ± 6.4 years), women performed better than men on mental flexibility as assessed by the Trail Making Test.[43] By contrast, men performed better than women on a test of visual processing assessed with a figure recognition task. Despite impairment in men and women, sex differences were not forthcoming on ability to overcome cognitive interference assessed with the Stroop Color and Word Test.[43]

Taken together, chronic excessive drinking in women is associated with myriad cognitive deficits, overlapping but not identical to the pattern of deficits observed in men. Although some evidence indicates that women develop cognitive deficits earlier in their disease or at lower lifetime consumption rates than men, its generalizability has not been clearly established.

POTENTIAL BENEFITS ASSOCIATED WITH MODERATE DRINKING

Despite the association of chronic excessive drinking with cognitive and motor deficits, much has been made about the potential beneficial health effects associated with moderate drinking—notably decreased risk of cardiovascular disease, better overall cognitive ability, and a slower rate of cognitive decline associated with normal aging.[44-47] Moderate drinking is generally defined as no more than one standard drink (14 grams of 95% alcohol) per day for women and two standard drinks per day for men. The pattern of performance from no drinking to excessive drinking has often been denoted as a U-shaped curve[48,49] or a J-shaped curve[50] with amount drunk modifying performance level.

Even moderate levels of alcohol consumption, however, have been associated with an increased risk of breast cancer, liver-related diseases, and cardiomyopathy in women (https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/women-and-alcohol), as well as infectious diseases, gastrointestinal disorders, and alcohol-related injuries.[51] In addition, for older women (particularly those age 60 and older), interactions between alcohol consumption at any level and aging, age-related disease, and drugs commonly prescribed to older people (including antibiotics, antidepressants, anxiolytics, and warfarin) can be hazardous.[52] Indeed, in addition to comorbid use of other drugs and medical comorbidities, AUD in older women often presents with complex clinical issues including untreated or undertreated depression and anxiety, which can exacerbate problems related to consumption and consequences of alcohol, family responsibilities, and feelings of guilt and shame surrounding their drinking. Although concern for older women in relation to

alcohol consumption is not new,[53] there remains a dearth of literature addressing the complexity of the factors associated with AUD in the elderly. With such a range of medical and mental health problems in this subpopulation, personalized treatment plans taking into account the entire picture and not just problem drinking are needed if abstinence and recovery are to be successful.[52]

An early study examining sex differences in 1,389 low to moderate drinkers (574 men, 815 women; ages 59 to 71) reported that women who were light (fewer than two drinks daily) to moderate (two or three but fewer than four drinks daily) drinkers performed better on set shifting, as assessed by the Trail Making Test, Part B, than women who reported abstaining from alcohol.[48] This beneficial effect of light to moderate drinking was not observed for men. These authors reiterated the importance of controlling for variables such as age, education, income, depressive symptoms, and smoking status in studies examining sex-related cognitive differences in relation to alcohol.

More recently, a longitudinal study of 818 older adults (age 65 and older; 139 moderate drinkers and 679 nondrinkers) found that although moderate alcohol use (defined as one to 14 drinks per week; average number of drinks per week in this cohort = 5.02 $\pm$ 3.79 *SD*) was related to higher baseline cognitive performance, no relation was observed on rate of change over time (spanning 7 years) across cognitive domains.[54] These authors highlighted the importance of future research focusing on the influence of demographic, genetic, and lifestyle factors on the variability observed in moderate drinking in relation to cognition. Indeed, another study cautioned that studies reporting beneficial effects of moderate drinking may have included an inappropriate selection of reference groups and little control for confounders.[55] The authors of this study found a beneficial dose-response relation only for women drinkers age 65 and older, with no measurable benefit of moderate drinking in other age-sex groups.

Another longitudinal study examined the relation between cognitively healthy longevity—defined as living to age 85 without cognitive impairment, as assessed by the Mini-Mental State Examination—and amount and frequency of alcohol intake in 1,344 older community-dwelling adults (728 women and 616 men; ages 55 to 84) and found a beneficial effect of regular, moderate drinking.[44] Indeed, individuals who reported drinking at moderate to heavy levels—up to three standard drinks per day for women on a near-daily basis—had twofold higher odds of living to age 85 without cognitive impairment compared with nondrinkers.[44] Nonetheless, another study of nondemented autonomously living octogenarians reported that older women who drank moderately did not appear to benefit at the same level as older men who drank moderately when it came to cognitive performance.[56] Indeed, only a relatively modest benefit in verbal memory for short stories was observed in women compared with men with moderate-level drinking. Sex differences were speculated to be due to myriad factors including drinking patterns and alcohol-related pharmacokinetics.

ALCOHOL CONSUMPTION AND RISK OF DEMENTIA

It is projected that the U.S. population age 65 and older will nearly double, from 48 million currently to 88 million by 2050 (https://www.nih.gov/news-events/news-releases/worlds-older-population-grows-dramatically). With an ever-increasing aging population, it is imperative to understand the effects of chronic excessive drinking on the structure and function of the aging brain and the moderating and mediating effects of age-related medical and psychiatric conditions, interactions with medications, and life-related stressors.

A meta-analytic study assessing risk of dementia in relation to alcohol consumption reported a modest U-shaped relation.[57] Results highlighted that moderate alcohol consumption, defined as fewer than 12.5 g/day (about one standard drink), was associated with a reduced risk of dementia, whereas drinking to excess (defined as $\geq$ 23 standard drinks per week) was associated with a significantly greater risk of dementia

compared with light drinking. The lowest risk of dementia was associated with drinking 6 g/day of alcohol, and wine was reported to be selectively associated with protective effects.

Another study—which included 2,874 women (of 9,087 total participants) with an average length of follow-up of 23 years—reported that abstainers and those who drank heavily (defined as more than 14 standard drinks per week) had a greater risk of dementia, determined from electronic health records.[58] These authors speculated that nondrinkers and those who drink excessively may be at higher risk of cardiometabolic disease including diabetes and hypertension, which, in turn, is associated with an increased risk of dementia.

At-risk drinking in the elderly is a timely issue. One study noted that 12% of older women (age 60 and older) reported drinking in excess of the recommended guidelines of no more than one standard drink a day or seven standard drinks per week but without meeting diagnostic criteria for AUD.[52] Without proper screening and intervention, these older adult women may be at particular risk for alcohol-related health and cognitive problems including dementia.

EMOTIONAL PROCESSING AND SOCIAL COGNITION IN WOMEN WITH AUD

Over the past decade, emotional processing and social cognition have become a focus of addiction research, highlighting the relevance of one's abilities to identify and respond to emotional and social cues in interpersonal interactions at home, at work, and with friends. Sex differences outside of AUD typically note better performance in women than men in decoding emotional facial expression and in performing tasks of social cognition such as the Reading the Mind in the Eyes Test or the Faux Pas Recognition Test.[59-63] Taken together, these findings suggest a potential resilience to social cognition disorders in women. This section reviews whether AUD disrupts this protective factor as a whole or interferes with selective processes.

AUD is associated with difficulties in components of emotion processing and social cognition, notably alexithymia, issues in decoding others' emotions, inferring others' mental states or feelings (i.e., Theory of Mind [ToM] deficit), and experiencing empathy.[64] Factors contributing to deficits in emotional processing and social cognition include an increased risk of personal, social, and work problems as well as poor initiation of action to achieve abstinence in AUD.[65] Vulnerability to emotional decoding and social cognition impairment in women with AUD may trigger an additional burden in their emotional and interpersonal interactions, thereby increasing relapse risk. Despite known sex differences in the severity of brain compromise and cognitive impairment in AUD,[66] the literature on sex differences in emotional processing and social cognition in AUD is scant.

Alexithymia is a multidimensional personality construct that comprises four core characteristics: (1) difficulty identifying feelings in oneself and differentiating feelings from the physical sensation of emotional arousal, (2) difficulty describing feelings to others, (3) restricted imaginative processes featured by limited fantasy life, and (4) an externally oriented style of thinking.[67] Alexithymia is commonly assessed by the Toronto Alexithymia Scale-20 (TAS-20), a self-report questionnaire, exploring three factors: difficulty identifying feelings, difficulty describing feelings, and externally oriented thinking (i.e., tendency to focus attention outside of oneself).[68] Higher prevalence of alexithymia in women with AUD than in men with AUD has been observed, especially on the global TAS-20 score and its "difficulty identifying feelings" factor.[69] Interestingly, alexithymia factors can play a moderator role in the relations between depressive mood and craving for alcohol in recently detoxified individuals with AUD.[70] In particular, women with AUD who reported difficulty describing feelings were at higher risk for craving when experiencing depressed mood, which is

consistent with the hypothesis that relapse would be more frequently associated with negative affect in women than men.[71]

Emotion decoding skills are crucial when assessing one's immediate social environment, providing valuable information regarding others' internal affective state, enabling behavioral adaptation according to others' thoughts and intentions, and facilitating social interactions in daily life. Contradictory findings on sex differences have been reported in studies that assessed decoding of emotional facial expressions (EFE) in AUD. Although no evidence of sex differences was found in recently detoxified individuals,[72,73] vulnerability to alcohol-related EFE recognition deficits was reported in recently detoxified women.[74,75] Lack of consistency between studies could be related to the small sample sizes of women (fewer than 15 women), which may not be representative of the population of women with AUD. Elsewhere, assessment with the social cognition module of the Wechsler Advanced Clinical Solutions revealed significant impairment in recognizing affect from facial expression in long-term abstinent men but not in long-term abstinent women.[76] Although the women did not differ from their sex-matched controls, better identification of emotional facial expressions was related to longer length of abstinence.

ToM refers to the ability to attribute mental states to oneself and others, and to understand that others' mental states might differ from those of oneself.[77] ToM enables individuals to predict, anticipate, and interpret the behavior of others and facilitates appropriate social interactions.[78] Large effect sizes were identified in two recent meta-analyses for deficits in ToM in AUD.[79,80] In support of the vulnerability hypothesis of emotional and social functioning impairment in women with AUD, a meta-analysis indicated that the effect size was modulated by sex, such that increasing the percentage of men in the treatment group decreased the effect size—results suggesting that "AUD is more likely to be associated with affective ToM deficits in females."[80(p 413)]

SEX DIFFERENCES IN ALCOHOL EFFECTS ON BRAIN STRUCTURE AND FUNCTION

Three decades of magnetic resonance imaging (MRI) studies describe patterns of brain structural abnormalities characteristic of chronic, heavy drinking.[81,82] Despite the rich literature on neuroimaging in AUD, the mainstay of studies does not address sex differences. The focus of this section is on the research in women with AUD and starts with studies using conventional structural MRI to quantify regional brain volumes; also summarized are studies using magnetic resonance diffusion tensor imaging to assess the microstructural integrity of white matter fibers and finally functional MRI done in the task activation state.

Structural MRI

Individuals with AUD but without neurological complications generally show ventricular expansion and shrinkage of selective cerebellar lobules and regions of the cerebral cortex. Volume deficits in cerebellar and cortical regions generally extend to gray and white matter macrostructure and microstructure. Whole-brain analyses support the profile of widespread damage to gray matter structures, including the frontal cortex, thalamus, insula, hippocampus, and cerebellum, as well as white matter regions including the cerebellar peduncles, pons, corpus callosum, and periventricular area.[83-87] The exploration of specific brain damage in women with AUD has been limited by an inclusion bias of men in most studies and by the lack of methodological consideration of sex differences with respect to an appropriate control group matched in sex and other relevant factors to the clinical group. The few neuroimaging studies considering differences between men and women on alcohol-related brain structural changes have generated conflicting results.

A number of cross-sectional studies investigating brain macrostructural abnormalities and alcohol misuse have reported no sex differences in brain volumes.[85,88] However, other

studies have reported inconsistent findings including greater vulnerability in men than women,[89,90] greater susceptibility to structural abnormalities in women than men,[91,92] and sex-related differences in the pattern and severity of regional brain volumetric deficits.[66] A study using a longitudinal design tested for, but did not find, sex differences on brain volumes related to chronic heavy drinking.[93]

Hippocampal volume deficits were identified in individuals with moderate alcohol consumption (fewer than 14 standard drinks per week for women, fewer than 21 standard drinks per week for men) in a study of 527 community-dwelling men and women who did not have AUD (mean age = 43 $\pm$ 5.4 years). This dose-dependent relation between alcohol consumption (i.e., alcohol units/week) over 30 years and hippocampal shrinkage, however, was significant only for men and not for women.[49] A lack of effect in women may be attributed to inadequate statistical power given the smaller number of women (n = 103) than men (n = 424) in the study and the fact that few women in the study were categorized as unsafe drinkers (n = 14 women reported drinking more than 14 standard drinks per week). In addition, no demonstrable beneficial effect was observed with light alcohol consumption compared with abstinence on brain structure and function. The authors cautioned that the protective effect reported in association with moderate drinking in other studies may be due to confounding variables, such as socioeconomic status or IQ. Beneficial effects, defined as a reduction of age-related decline in brain volume, also were not observed in a study of nondependent (DSM-IV) drinking men and women, with a relation between greater amount of alcohol consumed and smaller total brain volume, which was more pronounced in women than men.[94]

Diffusion Tensor Imaging (DTI)

This neuroimaging approach enables examination of the integrity of the microstructure of white matter, which comprises linearly organized fiber tracts that connect proximal and distal gray matter regions (that is, brain structures composed of neurons). Fiber integrity is measured in terms of fractional anisotropy (FA), typically higher in fibers with a homogeneous or linear structure such as healthy white matter, and bulk mean diffusivity of water movement for which higher values reflect diminished integrity or edematous tissue. In men with AUD, the greatest microstructural white matter abnormalities are reported in the corpus callosum, but for women with AUD, these abnormalities are greatest in the centrum semiovale.[95] In other cross-sectional DTI studies, when matched for alcohol history variables, women with AUD showed more signs of white matter degradation than men with AUD in several fiber bundles, suggesting an enhanced risk for alcohol-related degradation in selective white matter systems.[96] By contrast, no evidence for alcohol-related sex differences was forthcoming in DTI metrics for six anatomically defined transcallosal white matter fiber bundles.[97]

Potential sex differences in brain structural recovery with abstinence require further investigation. Contradictory results based on relations with length of abstinence[66,98] showed stronger positive association between length of sobriety and white matter volumes in women with AUD than in men with AUD within the first year of abstinence.[66] By contrast, positive associations between length of sobriety and white matter volumes were observed in men with AUD but not in women with AUD after 1 year of abstinence, suggesting faster white matter recovery in women.

Another DTI study reported relations between longer duration of abstinence and higher FA of the callosal white matter in men with AUD, but not in women with AUD.[98] The authors suggested better callosal white matter recovery with abstinence in men, especially when men with shorter length of abstinence showed lower FA than recently abstinent women, but the opposite pattern was observed for longer duration of abstinence. Moreover, recent neuroimaging investigations found sex interactions displaying opposite patterns. Compared with control men, men with AUD had smaller volumes in the reward network

and lower FA in select white matter tracts. By contrast, women with AUD had larger volumes in the reward system and higher FA in the same white matter tracts compared with control women.[98-100] These authors suggested that this opposite pattern in brain structural abnormalities between men and women with AUD might reflect a sex-specific phenotype related to dissimilarities in neuroanatomical and neurobehavioral expressions as risk factors or in sex-based motivation to seek alcohol.

Functional MRI

The literature investigating sex-related effects on brain functioning in AUD with functional MRI (fMRI) is scarce and is sampled next. A task-activated fMRI study revealed lower brain activation in the prefrontal and parietal cortices during a spatial working memory task in 10 women with AUD compared to 10 healthy women controls.[101] During high-risk decisions to drink, control women activated the default mode network, whereas women with AUD simultaneously activated the reward, cognitive control, and default mode networks. These results suggest that risky decisions to drink could be associated with difficulties to switch between different neural networks in women with AUD, potentially due to dysfunction in the anterior insula.[102]

A small fMRI study of airplane pilots—individuals with AUD (8 women, 6 men) and healthy controls (9 women, 5 men)—revealed an interactive effect of AUD and sex on brain activation during negative and positive facial affective processing, such that men with AUD demonstrated higher brain activation than control men, whereas women with AUD showed lower brain activation than control women.[103] By contrast, an fMRI study conducted in long-term abstinent individuals with AUD reported sex-related differences in the pattern of brain responsivity to emotional stimuli, with lower activation in the rostral middle and superior frontal cortex, precentral gyrus, and inferior parietal cortex in men with AUD than in control men, whereas higher activation in superior frontal and supramarginal cortices were observed in women with AUD compared to control women.[104] As suggested, these specificities in brain reactivity between men and women during emotional processing may reflect sex-related differences in the emotional mechanisms leading to the development of AUD.

Taken together, these studies demonstrate the relation between chronic heavy drinking and structural and functional brain abnormalities in men and women; however, due to their cross-sectional nature, these studies cannot determine whether AUD-related brain dysmorphology was caused by drinking, was pre-existing, or both. Prospective longitudinal studies—such as the National Institutes of Health/NIAAA-supported National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA)[105] and the Collaborative Studies on the Genetics of Alcoholism (COGA)[106]—study adolescents before they initiate appreciable drinking. Assessing children as young as age 8, the Adolescent Brain Cognitive Development (ABCD) Study is a longitudinal prospective study[107] that aims to identify the antecedent and resultant effects of alcohol and to track the drinking patterns that contribute to deviations from normal neurodevelopmental growth trajectories in cerebral[108] and cerebellar[109] volumes starting in preadolescence. These studies also will provide information that can address questions of specific sex-related risk factors that contribute to excessive drinking behavior and underlie differential prodromal brain abnormalities between men and women with AUD.

RECOVERY OF COGNITIVE ABILITIES WITH SUSTAINED ABSTINENCE

On an optimistic note, potential for recovery of selective cognitive deficits including memory and psychomotor abilities can occur with sustained abstinence. Functions that appear more resistant to recovery include visuospatial skills and gait and balance stability, which often endure

even with long-term abstinence.[110-113] Cognitive impairment has been associated with higher rate of relapse and lower motivation to initiate and maintain abstinence.[114]

One of the earliest studies examining recovery of cognitive function with abstinence included both short-term abstinent (1 month, $n = 40$) and long-term abstinent (4 years, $n = 40$) women.[115] This study indicated differential recovery among cognitive processes, with long-term sober women showing improvement on complex tasks of abstraction, assessed with the Halstead Category Test, whereas perceptuomotor ability, assessed with the Digit Symbol Test and the Trail Making Test, Part A, was more resistant to recovery. Critically, it was the subset of women who resumed drinking after baseline assessment that accounted for the greatest deficits at baseline compared with the subset of alcoholic women who remained sober. These authors highlighted the possibility that heterogeneity within their cohort could partly be explained by difference in posttreatment drinking (resumers vs. abstainers) and by differential premorbid "at-risk" variables in women compared with men with AUD.

Follow-up of a cohort of women with AUD at 3 to 6 years post–baseline testing after an average of 3 months of sobriety[41] reported recovery of nonverbal short-term memory and psychomotor speed.[111] Postural instability, however, was still noted, even after this extended length of abstinence. These studies highlight the selectivity of dissociable cognitive and motor processes in terms of time course and extent of recovery with abstinence.

An investigation of cognitive recovery after 6-week sobriety in a controlled environment after being in a residential treatment unit reported that a slightly lower percentage of women than men (41% vs. 46%) showed recovery on a general cognitive measure.[116] These authors speculated that the timeline of recovery and factors promoting recovery may differ between men and women and highlighted the relevance of examining the effect of sex on remediation and extent and the timeline of recovery of component cognitive processes.

FACTORS THAT MODERATE OR MEDIATE COGNITIVE AND MOTOR PERFORMANCE IN WOMEN WITH AUD

Hormonal differences between men and women and within cohorts of women have been hypothesized to at least partially underlie sex differences reported in AUD, although studies to establish this relation have been inconsistent and inconclusive.[9,117] Only limited evidence suggests that phase of menstrual cycle accounts for a significant amount of the variability in behavioral response to alcohol, with a number of studies finding that phase of menstrual cycle had no significant effects on alcohol consumption in women.[117,118] In addition, no differences among menstrual phases in alcohol pharmacokinetics have been forthcoming.[119]

Other factors speculated to moderate or mediate cognitive performance between alcoholic men and women or to underlie the heterogeneity among women with AUD are (1) age and aging effects and their interaction with alcohol; (2) alcohol consumption variables including age of AUD onset, amount drunk in one's lifetime, quantity and pattern of binge events, family history of alcohol misuse, and number and severity of withdrawals; (3) nutritional status including thiamine and other vitamin B deficiencies; (4) existence of comorbid medical and health conditions including HIV, hepatitis C, and chronic pain; (5) other drug use (including prescription and illicit); and (6) psychiatric symptoms and disorders.[37,65,120]

Research strongly supports the notion that whether one maintains sobriety or relapses into drinking, even when drinking does not meet AUD criteria, may moderate the extent and rate of cognitive and motor recovery in AUD. Attention has been paid recently to the history of trauma and chronic pain and their relation to initiation and maintenance of hazardous drinking in women and bidirectional effects of alcohol on these factors.[120,121]

Pain, for example, may be both a risk factor and a consequence of excessive drinking.[121,122] Although alcohol can reduce and even quell pain in some individuals when alcohol is initially used, over time increasing amounts of alcohol are needed to achieve pain relief, with the paradoxical effect that alcohol consumption exacerbates pain intensity. In a study of 451 treatment-seeking participants with an alcohol misuse diagnosis in residential treatment, women were more likely to report significant recurrent pain, more concurrent chronic pain conditions, and greater pain severity than men.[122] Taken together, these studies highlight the relevance of including effective pain management in initiation and maintenance of abstinence, particularly in women.

LIMITATIONS OF STUDIES

Limitations commonly noted in studies on the cognitive effects associated with chronic excessive drinking include the fact that most of the data pertaining to alcohol consumption variables, including pattern, severity, and amount, are obtained through self-report. Structured follow-back interviews likely aid accuracy of documentation but are subject to memory distortion. Differences in subject inclusion and exclusion criteria and task demands make it difficult to generalize across studies; standardization of participant characteristics and tests would allow meta-analyses across data. Additionally, the dearth of longitudinal reports limits the ability to determine whether a deficit was pre-existing or caused by alcohol misuse or to document the temporal sequence of cognitive declines and recovery in relation to the dynamic nature of alcohol use.

Additional limitations relevant to review of studies on moderate alcohol consumption and cognition and women include inclusion of "sick quitters" in the group of abstainers—that is, individuals who no longer drink because of previous alcohol misuse.[51] Efforts were taken to include studies where this was not a clear issue. Further, this review only included studies assessing sex differences and not gender differences, per se.

TREATMENT IMPLICATIONS AND CONCLUSION

There is a growing appreciation of direct comparisons between men and women in the examination of alcohol's effects on brain structure and function and the identification of factors contributing to alcohol-related cognitive impairment, including those that affect personal, social, and professional lives. Of course, regardless of sex, assessment of cognitive deficits is relevant to treatment plans, as it has been documented that efficacy of treatment with a heavy cognitive behavioral therapy component may be best delayed until recovery of the cognitive processes relevant to task demands.[123]

Highlighting the cognitive effects of acute, moderate, at-risk, and excessive drinking in women speaks to the urgency of screening, treating, and monitoring women who report patterns of possible alcohol misuse, even if diagnostic criteria for AUD are not met.[124] Young adults should be educated on the cognitive effects of binge and intensive drinking for both the short term and the long term.[125] Older adult women need to be educated on how alcohol interacts with age-related biological changes, comorbid medical conditions related to aging, and medications.

Longitudinal studies that examine the pattern and extent of cognitive and motor deficits associated with chronic heavy drinking and the factors that play a role in initiation and maintenance of alcohol misuse will continue to have both theoretical and clinical implications, steering specialized treatment for women with AUD and informing practice and policy. Heterogeneity among women with AUD highlights the complexity of this chronic disease and underscores the relevance of examining the effects of demographic factors, especially age and aging factors, and disease-related variables, notably

pattern of drinking and duration of abstinence, in identifying the cognitive effects of alcohol and its biological underpinnings.

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Sleep and Alcohol Use in Women

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Sleep disturbance is common among individuals with alcohol use disorder (AUD). Insomnia not only is a pathway toward alcohol consumption but also is related to increased risk of relapse, psychosocial impairment, decreased quality of life, and suicidal ideation in individuals with AUD. Few studies examining sleep disturbance and alcohol use have explored how this relationship differs between men and women. Historically, studies of AUD have included few, if any, women in their samples. However, women are increasingly consuming alcohol at an earlier age and at higher rates, and the effect of alcohol on women's mental and physical health is expected to rise. This narrative review consolidates findings from studies that have reported the effects of acute and chronic alcohol use on sleep among women. Additional research is needed to investigate sex differences in this area. Such research should consider the modifying effects of age, lifetime alcohol use, and psychiatric co-occurrence, as well as the effectiveness of combined interventions for AUD and sleep disturbance.

KEY WORDS: adolescence; alcohol use disorder; circadian; sex differences; slow wave sleep; substance use

INTRODUCTION

Sleep disturbance is one of the most common complaints of individuals with alcohol use disorder (AUD), with prevalence estimates ranging from 36% to 91%.[1] Insomnia in particular has been associated with multiple aspects of AUD: relapse to drinking, psychosocial impairment (e.g., employment problems, social conflict, and impulse control), decreased quality of life, suicidal ideation, and insufficient sleep duration. (For

definitions of insomnia and other technical terms, see the box **Glossary of Sleep Terms.**) Sleep disturbance can serve as a pathway to increased alcohol use, in part because alcohol can be used as a sleep aid to reduce time to sleep onset. However, even acute alcohol consumption increases sleep disruption throughout the night, and tolerance to the sedating qualities of alcohol accumulates quickly.[2] In people with AUD, chronic alcohol use is related to changes in sleep structure that persist into abstinence. For abstinent individuals with AUD, this persistent sleep disturbance is a risk factor for relapse.[1] Once relapse occurs, the cycle repeats, as continued consumption of alcohol perpetuates sleep disturbance.

Historically, studies of AUD and sleep have mostly included men. Although women with AUD have been recruited for a handful of studies,[3-7] women have largely been underrepresented in the research that examines the relationship between sleep and alcohol use. Sex differences in the effects of alcohol are dependent on the interaction of many biopsychosocial factors. Sleep intertwines with several of these relationships: alcohol disrupts sleep, and sleep disturbance relates to increased risk of psychiatric co-occurrence, alcohol misuse, and relapse to AUD. In addition, sleep is a modifiable behavior.[8,9] Thus, understanding how sleep problems relate to problematic alcohol use and the extent to which this relationship differs between men and women can inform the development of targeted methods for prevention and treatment of AUD.

This narrative review aims to stimulate new research in this area by consolidating findings from studies that have reported effects of acute and chronic use of alcohol on sleep among women. First, an overview of sex differences in sleep disorders is provided, followed by considerations for how sex may modify the relationship between alcohol use and sleep. (For consistency, both biological and psychological/sociological/cultural factors are referred to as "sex"-related throughout the review.) The review concludes by providing treatment considerations and directions for future research.

SEX DIFFERENCES IN SLEEP

Sleep is a universal process across species and is a behavioral state that is essential to physical and mental health in humans. Changes in brain activity throughout the night demarcate different stages of sleep. This neuronal activity, along with muscle activity and eye movements, can be measured via polysomnography (PSG) to provide an objective measure of sleep. Sleep is divided into stages (N1, N2, and N3) of non–rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep.[10] Throughout the night, sleep follows a cyclical pattern. Each cycle begins with stage N1, and the majority of time is spent in stage N2 before progression to stage N3 (deep sleep) and eventually to REM sleep. Each cycle lasts approximately 90 minutes. More detailed analysis of the sleep electroencephalogram (EEG) is possible with spectral analysis to determine activity during sleep within a specific frequency band (e.g., slow wave activity).

PSG provides a detailed, objective measure of sleep architecture and quality but is mainly confined to the laboratory. Actigraphy (usually measured with devices worn on the wrist) relies on an accelerometer to measure patterns of activity from which sleep–wake states can be estimated.[11] Actigraphy is useful for objective assessments of sleep outside the laboratory environment. Self-perception of sleep quality is also valuable and can be measured over many nights with questionnaires or sleep diaries.

Glossary of Sleep Terms

Actigraphy: An objective measure of sleep quantity and circadian patterns that uses an accelerometer (generally worn like a wristwatch) to detect sleep–wake activity over several days or weeks.

Apnea-hypopnea index: An index used to indicate the severity of sleep apnea that is represented by the number of apnea and hypopnea events per hour of sleep.

Circadian period: The amount of time for a cyclical process to return to the same phase (e.g., from one day's waking to the next day's waking).

Circadian preference/ chronotype: An individual's tendency towards relatively earlier or relatively later sleep and activity patterns, typically measured via preferred timing (i.e., morningness versus eveningness) or self-reported actual timing (i.e., early versus late chronotype).

Circadian rhythm: An endogenous 24-hour rhythm, typically measured via levels of melatonin or by core body temperature.

Circadian timing: The timing of biological processes that follow a circadian rhythm (e.g., sleepiness, wakefulness, melatonin, body temperature).

Hypopnea: The partial blockage of air, resulting in decreased airflow and oxygen saturation.

Insomnia: A sleep disorder characterized by difficulty falling asleep or staying asleep, causing distress or impairment in daytime functioning.

K-complex: A high-voltage delta frequency EEG event seen in NREM sleep that occurs when large numbers of healthy neurons fire in a synchronized manner.

Non–rapid eye movement (NREM) sleep: The sleep stage characterized by slower, higher amplitude EEG activity, regular breathing and heart rate, muscle tone (i.e., low-level contraction), and a lack of eye movement; consists of stages N1, N2, and N3.

Polysomnography (PSG): A test conducted to study sleep and diagnose sleep disorders using a multitude of physiological measures, including measures of brain activity, blood oxygen levels, heart rate, breathing, and muscle movements.

Rapid eye movement (REM) sleep: The sleep stage characterized by low-amplitude, high-frequency EEG activity, rapid eye movement, irregular respiration and heart rate, and muscle atonia.

Sleep apnea: A sleep disorder in which breathing is repeatedly interrupted during sleep.

Sleep architecture: The structural organization of sleep, such as cyclical alternation of NREM and REM sleep stages.

Sleep behavior: Self-report measures from questionnaires that typically ask about sleep over a period of weeks or months.

Sleep-disordered breathing: An umbrella term that encompasses breathing disorders and respiratory abnormalities that occur during sleep, including sleep apnea and snoring.

Sleep efficiency: The total number of minutes of sleep divided by the number of minutes in bed.

Sleep electroencephalogram (EEG): A recording of brain activity during sleep.

Sleep onset latency: The number of minutes to fall asleep after the lights are turned off.

Sleep timing: The times of day an individual goes to sleep and wakes up.

Slow wave activity: EEG activity in the delta (slow wave) band (0.5 Hz to 4.0 Hz), typically averaged separately for NREM and REM sleep for the entire night.

Slow wave sleep: The deepest stage of NREM sleep (stage N3), characterized by more than 20% delta wave EEG activity.

Stage N1: The lightest stage of sleep, which occurs right after falling asleep; characterized by low-voltage, fast EEG activity.

Stage N2: The intermediate stage of sleep that follows stage N1; characterized by theta activity (4-7 Hz), K-complexes, and bursts of faster activity on EEG.

Stage N3: The deepest stage of sleep; characterized by high-amplitude slow waves on EEG.

Total sleep time: The total number of minutes asleep.

Total wake time: The total number of minutes awake during the sleep period.

Wake after sleep onset: The number of minutes awake after falling asleep.

Differences in Sleep Measures

Women tend to have better sleep quality, as measured by PSG, than men. Women have less total wake time, shorter sleep onset latency, better sleep efficiency, and a larger percentage of slow wave sleep and slow wave activity (for definitions of these sleep measurements, see the box **Glossary of Sleep Terms**).[12] The prevalence of sleep-disordered breathing is 9% among women versus 24% in men. However, women with sleep-disordered breathing are more likely to present with initial symptoms of insomnia or fatigue rather than the typical symptoms associated with sleep-disordered breathing, such as snoring, daytime sleepiness, and witnessed apneic events.[13]

Although PSG is considered the gold standard of sleep measurement, it has limitations. PSG cannot capture habitual sleep duration under naturalistic settings and may miss subcortical brain activity (particularly in regions shown to be involved in conscious awareness) that may be more prominent in individuals with insomnia than in those who sleep well.[14] Although not yet examined, possible sex differences in subcortical brain activity during sleep may explain the finding that women report poorer subjective sleep quality than men despite having better PSG-based sleep quality.

When using subjective measures, women report more sleep problems than men, including disrupted and insufficient sleep, poor sleep quality, difficulty falling asleep, frequent night awakenings, and time awake during the night.[15,16] Women also have a 40% greater risk of insomnia[12] and report earlier sleep timing (i.e., bedtime and wake time) than men.[17] Potential reasons for sex differences in sleep are described briefly in this review. For more detailed discussions, see the reviews by Mong and Cusmano[12] and Krishnan and Collop.[13]

Biological Differences

Sex steroids (i.e., testosterone in men and estrogen and progestins in women) modulate sleep differently. Generally, women's sleep is more sensitive to changes in ovarian steroids.[12] For example, sex hormones modulate the orexin/hypocretin system, which plays an important part in regulating sleep and wake states.[18] Therefore, fluctuations in ovarian steroids in women (e.g., puberty, menstrual cycle, menopausal transition) are associated with changes in sleep and circadian rhythms[19] and increased prevalence of sleep disturbance.[20,21] In addition, among men and women with similar sleep timing and duration, women have a shorter circadian period and earlier circadian timing of endogenous temperature and melatonin rhythms.[12] (For definitions of these circadian terms, see the box **Glossary of Sleep Terms.**) This mismatch in sleep timing and circadian timing can cause sleep disturbance, such as problems with sleep maintenance and/or early morning awakening, which, in part, may underlie women's increased risk for insomnia.

Psychosocial Differences

Among women, those with more anxiety and more perceived nighttime awakenings also report worse subjective sleep quality, despite a lack of objectively measured sleep disturbance.[12] Anxiety and depression are both more prevalent among women and are strongly associated with insomnia. The risk of affective disorders increases at the onset of puberty, especially among girls.[22]

ALCOHOL AND SLEEP

Sex differences occur in sleep continuity and sleep architecture measures as well as in the prevalence of sleep disorders like insomnia and obstructive sleep apnea. Sex differences also have been reported in alcohol use patterns, biological effects of alcohol, and risk factors for heavy alcohol use. Alcohol use likely affects sleep systems differently in men and women, and pathways that link sleep disturbances with subsequent heavy alcohol use also may differ according to sex. In this section, we review the evidence for sex differences in bidirectional relationships between sleep quality and alcohol use (although directionality is not always clear when based on findings from observational or cross-sectional studies).

Sleep and wake states are regulated by complex patterns of neurotransmitter release and

neural activation, many of which are affected by alcohol.[23] Individuals who have trouble sleeping may initiate alcohol use as a sleep aid. Because alcohol affects the gamma-aminobutyric acid (GABA) neurotransmitter system, alcohol acts as a sedative and reduces time to sleep onset, increases slow wave sleep, and suppresses REM sleep in the first half of the night.

Alcohol has acute neurotoxic effects that affect receptors important for sleep generation. As alcohol metabolizes (at 7 grams per hour, on average), its sedating benefits diminish.[24] Later in the night, sleep becomes more disrupted and awakenings are more frequent. Thus, the effects of alcohol on sleep differ depending on which half of the night is examined. Chronic alcohol exposure damages nerve cells and fibers, reducing the likelihood of synchronized neuronal firing across the cortex, which is necessary for slow wave sleep. With prolonged use, neurotransmitter systems adapt and modulate their release, which can increase sleep disruption and change sleep architecture, sometimes permanently.[23,25]

Studies (mostly among men) indicate that these changes in sleep structure persist during abstinence, and disturbed sleep is a risk factor for relapse.[1] Therefore, sleep disturbance has been suggested as a target for treatment, potentially decreasing the risk of problematic alcohol use while also increasing the likelihood of abstinence.

Sleep Architecture

This section examines studies (which included women participants) of both the acute and chronic effects of alcohol on sleep architecture. To the extent possible, results from experimental studies are emphasized.

Effects of acute alcohol use

First, we present studies that primarily used PSG to examine the acute effects of alcohol on sleep architecture. These experiments provide some evidence of directionality in the relationship between alcohol use and subsequent sleep quality. One of the first studies to investigate the effect of acute alcohol use on sleep, specifically in young women, was conducted by Williams and colleagues.[26] As part of this double-blind trial, 11 healthy women (ages 18 to 21) completed several nights of PSG an hour after consuming a beverage with either 0.00, 0.50, or 0.75 grams of alcohol per kilogram of body weight (g/kg). Results were consistent with previous findings reported for men. As the alcohol dose increased, sleep onset latency decreased. A significant decrease in the percentage of REM sleep was found, which was most apparent in the first 3 hours of the night. Also, a dose-dependent increase in slow wave sleep during the first half of the night was found, followed by a decrease in slow wave sleep in the second half of the night. Furthermore, these women demonstrated a dose-dependent increase in the percentage of stage N1 sleep, with increased minutes spent in stage N1 sleep in the second half of the night.

A later study conducted by Van Reen and colleagues examined the extent that a moderate dose of alcohol (0.49 g/kg), compared to placebo, consumed an hour before bedtime affected the sleep and sleep EEG of 7 women (ages 22 to 25).[27] Similar to the findings reported for men,[23] this study reported that alcohol consumption led to an increase in slow wave sleep (in the first 2 hours) and an overall decrease in REM sleep.[27] Also, frontal EEG power during NREM sleep in the alpha range (9 to 11 Hz) increased relative to placebo following alcohol consumption.

In a direct evaluation of sex differences, Arnedt and colleagues performed PSG for 93 healthy adults (ages 21 to 31, 59 were female) following alcohol intoxication.[28] For this double-blind, randomized trial, all participants received alcohol on one night and placebo on another night, 1 week apart. Participants were given either placebo or alcohol (1.2 g⁄kg for men and 1.1 g⁄kg for women) 1 to 2.5 hours before bed. The alcohol dose was adjusted for weight and sex such that breath alcohol concentration (BrAC) levels were equivalent in men and women. At bedtime on the alcohol night, women reported higher ratings of sleepiness than men. Despite reaching equivalent BrACs, sleep continuity was more disrupted in women than in

men. For women, the total sleep time decreased by 20 minutes relative to the placebo night, and the wake after sleep onset time increased by 15 minutes. In addition, among women participants, the frequency of awakenings increased, and overall sleep efficiency decreased by 4% after alcohol intoxication. In men, no significant differences in sleep continuity measures (i.e., sleep onset latency, total sleep time, sleep efficiency, frequency of nighttime awakenings, and wake after sleep onset) between the placebo and alcohol conditions were reported. For both sexes, sleep architecture variables differed for the alcohol condition compared to the placebo condition—alcohol use increased slow wave sleep and decreased REM sleep.

Chan and colleagues also examined the effects of acute alcohol consumption (a mean dose of 0.828 g/kg an hour before bedtime) on the sleep architecture of 24 older adolescents (ages 18 to 21, 12 were female).[29] They found main effects of alcohol on sleep, dependent on halves of the night. In the first half of the night, participants experienced fewer arousals, less stage N1 sleep, increased slow wave sleep, and reduced REM sleep. In the second half of the night, they experienced less sleep efficiency and more time awake after sleep onset. These researchers did not find evidence for an interaction between sex and alcohol.

Effects of chronic alcohol use

The following studies are observational, such that they examine sleep among individuals with a history of chronic alcohol use in the context of many other variables. Individuals in these studies vary regarding the duration of their abstinence at the time of study, their co-occurring disorders, and their lifetime alcohol use. When participants were examined early (at less than 1 month) during recovery, the effects on sleep may have reflected the effects of withdrawal more than any chronic effects of heavy alcohol use. When participants were examined later during recovery, withdrawal effects would have subsided. Therefore, the associations observed do not prove causality in these relationships, but they provide a starting point to stimulate further research that may better distinguish directionality.

Colrain and colleagues collected sleep architecture and EEG measures from 42 abstinent participants (mean age of 49, 15 were women) with long-term AUD and from 42 control participants (mean age of 51, 23 were women).[5] Overall, women had better sleep efficiency, fewer periods of in-bed awake time, and more slow wave activity during NREM sleep than men. There were main effects of AUD for some sleep measures. For example, individuals with AUD had less slow wave sleep and slow wave activity during NREM sleep and more stage N1 and REM sleep than controls.

Despite a lack of significant interaction between sex and diagnosis, women with AUD and women control participants had similar amounts of NREM slow wave activity, whereas men with AUD had substantially lower NREM slow wave activity than men control participants.[5] Women with AUD had lower levels of lifetime alcohol consumption and longer periods of sobriety when compared with the men who had AUD in this study. Although greater estimated lifetime alcohol consumption was related to a lower percentage of slow wave sleep in men, this measure was not related to the percentage of slow wave sleep in women. This study did not investigate sex interaction effects, and the samples of women and men with AUD were unequal sizes, had varying lengths of sobriety, and had different levels of lifetime alcohol exposure.

Using the same sample, Colrain and colleagues examined K-complex incidence and amplitude during sleep.[6] K-complexes are high-voltage, delta frequency events that occur during NREM sleep when large numbers of healthy neurons fire together at the same time. They provide a sensitive measure of typical, healthy, brain aging. In this study, participants with AUD had both reduced K-complex incidence and amplitude. Men and women also showed the same pattern of AUD-related change in K-complex amplitude, despite women having less lifetime alcohol consumption.

In a sample that included 26 participants (ages 32 to 63, 10 were women) with alcohol dependence who were in subacute withdrawal from alcohol and 23 control participants (ages 24 to 61, 9 were women), overall, women spent a larger proportion of time awake during the sleep period, and they had shorter time to REM sleep.[7] The relationships between sleep parameters and group did not vary by sex; however, this analysis may have been underpowered because of the sample size. The investigators noted that the distribution of sex across groups was not equal.

A population-based study of sleep among 400 Swedish women (ages 20 to 70) found that women who self-reported alcohol dependence had longer sleep onset latency, reduced REM sleep, and more stage N2 sleep compared to women who did not report alcohol dependence.[30] In addition, alcohol dependence was related to decreased time spent in REM sleep and increased sleep onset latency, independent of age, body mass index, apnea-hypopnea index, smoking, and hypertension.

Summary

Sleep is a complex neurological function, and the extent that it may be affected after a single night of alcohol compared to chronic alcohol misuse can differ. Thus, sex differences in the acute effects of alcohol may not necessarily coincide with sex differences in the chronic effects of alcohol. The single experimental study that examined sex differences in the effect of acute alcohol consumption found sex differences in objectively measured sleep among healthy subjects (with equivalent BrAC levels before sleep), with women showing more disrupted sleep than men.[28]

Sex differences in alcohol pharmacokinetics may underlie these differences. Even at equivalent starting points, BrAC levels decline more rapidly for women than for men.[28] As alcohol metabolizes, alcohol metabolites disrupt sleep. Chronic alcohol misuse leads to changes in brain macrostructure and microstructure that can manifest as sleep disturbance.[25] Few studies have examined sleep in both men and women during recovery from AUD, and those studies have not had sample sizes large enough to statistically examine sex differences.

Further study is needed to examine potential sex differences in sleep among individuals with AUD who are abstinent. Dose effects, time in recovery, and the effects of interaction between age and sex should be considered. Sleep structure changes across age, and these changes vary by sex.[31] For example, women have a greater amount of slow wave activity than men, and although men tend to show a decrease in slow wave activity with age, women do not show the same pattern of decline.[12]

Sleep Physiology

Limited experimental work has examined whether the effects of alcohol on the functioning of physiological systems (e.g., respiratory or cardiovascular) during sleep differ according to sex.

Effects of acute alcohol use

In an investigation of the acute effects of alcohol, Block and colleagues monitored breathing and oxygenation during sleep for 78 participants (20 were men ages 20 to 40 years, 20 were men ages 40 years and older, 20 were women ages 20 to 40, and 18 were postmenopausal women ages 51 to 66) following consumption of 2 milliliters of alcohol per kilogram of body weight.[32] Men in both groups had more oxygen desaturation episodes across the night and greater severity of desaturation, but no effect of alcohol on breathing or oxygenation was found for either group of women. As expected, postmenopausal women had significantly more episodes of apnea and oxygen desaturation than premenopausal women, although this difference was unrelated to alcohol consumption.

A large, observational study of 1,420 men and women (mean age of 51, 645 were women) demonstrated similar findings.[33] Men showed increased likelihood of sleep-disordered breathing for each drink consumed per day (measured via a self-report questionnaire), whereas no association between minimal to moderate alcohol consumption and sleep-disordered breathing

was found for women. The investigators posited that circulating progesterone may protect young women in particular from the depressant effects of alcohol and consequent sleep apnea and oxygen desaturation,[32,34] and that hormonally mediated increased ventilatory drive and anatomical differences may also protect women from sleep-disordered breathing events.[33,35,36] Since alcohol had no effect on breathing for postmenopausal women, other nonhormonal factors may have played a role in the sex differences related to sleep-disordered breathing and alcohol consumption.

Effects of chronic alcohol use

A study of 24 patients with chronic AUD who were recently abstinent (10 were women ages 25 to 58) compared with 24 control participants (10 were women ages 25 to 58) showed that both males and females with AUD had a high number of observed apneic/hypopneic episodes, and this result did not differ by sex.[37] The researchers concluded that women with AUD were as likely as men with AUD to have a sleep-related breathing disorder.

In a study investigating autonomic nervous system functioning during sleep, de Zambotti and colleagues found that patients with AUD who were recently sober (n = 14, 7 were women ages 28 to 54) compared with healthy control participants (n = 16, 8 were women ages 30 to 62) had elevated heart rates, reduced total heart rate variability, and reduced high-frequency activity (a measure of vagal functioning) across the night.[4] Together, this pattern of findings indicates disrupted autonomic nervous system functioning during the night, providing compelling evidence of impaired cardiovascular functioning during sleep. Effects did not differ by sex, and women with AUD, despite having less lifetime alcohol consumption, were affected to the same extent as men with AUD. In a follow-up investigation across the first few months of abstinence, as the duration of abstinence increased, individuals with AUD showed substantial recovery in heart rate and vagal functioning during sleep, although examination of any modifying effect by sex was not possible in this small sample.[3]

Periodic limb movements can also contribute to disturbed sleep. Aldrich and Shipley found that periodic limb movements were more likely to occur at a clinically significant frequency among adults ages 19 to 81 who self-reported consuming 2 or more drinks per day (heavy users, n = 112, 24 were women) when compared with adults who consumed less than 2 drinks per day (abstainers and light to moderate users, n = 872, 317 were women).[38] In addition, women who were heavy users were more likely to report symptoms of periodic limb movements than women who were light users, whereas no difference was observed between the two groups of men.

Summary

For physiological measures, the evidence from one large, experimental study suggests that acute alcohol consumption does not affect women's breathing during sleep to the same extent it does for men, who demonstrate more oxygen desaturation events during the night. Also, among men, self-reported alcohol use is positively associated with greater likelihood of sleep-disordered breathing, although this relationship is not observed in women. However, women with AUD are just as likely as men to have sleep-disordered breathing.[37]

Women may be more susceptible to periodic limb movements, and alcohol use could be a potential trigger of these movements. Also, women who experience periodic limb movements may self-medicate with alcohol. One study with a small sample size suggested that chronic alcohol use may affect cardiovascular functioning in women more than it does in men, as women and men did not differ in these measures despite women having less lifetime alcohol consumption.

These results are consistent with other studies that have demonstrated that women are at greater risk of alcohol-induced cardiomyopathy and peripheral neuropathy despite fewer years of drinking and lower quantities of alcohol consumption.[39] Given that two of these studies examined men and women early during their recovery,[4,37] some of the effects found could reflect

residual withdrawal effects of alcohol. Further longitudinal studies across a period of recovery among men and women with AUD are needed to separate effects of alcohol withdrawal and chronic heavy alcohol use on sleep as well as on physiological measurements taken during sleep.

Self-Reported Sleep Behavior

Many individuals report using alcohol as a sleep aid,[40,41] even though the use of alcohol to help initiate sleep can further perpetuate sleep disturbance. In women older than age 60, using alcohol to sleep and shorter sleep onset latency each are associated with greater risk for alcohol misuse.[42] However, moderate alcohol use is associated with fewer insomnia symptoms in women, but not in men, older than age 65.[43]

In a study of healthy men and women, self-reported insomnia symptoms at baseline were associated with greater odds of heavy drinking at a 5-year follow-up.[44] Likewise, heavy drinking and binge drinking at baseline were associated with greater odds of insomnia symptoms at a 5-year follow-up. Although results specific to sex were not reported, the investigators noted that these associations were similar among men and women but reached statistical significance only for women.

Some epidemiological studies have considered associations between alcohol use and insomnia symptoms among women in midlife and after menopause, an age group in which sleep problems are common. Blümel and colleagues reported that troublesome drinking (assessed with the Brief Scale of Abnormal Drinking) in a group of women ages 40 to 59 was strongly associated with increased risk for insomnia symptoms more than other factors, including mood and vasomotor symptoms, education level, and use of hypnotics.[45] In contrast, frequency of alcohol use (i.e., not currently, occasionally, or regularly in the past week) was not associated with sleep disturbances in a group of postmenopausal women ($N = 322$, ages 60 to 70).[46] These findings show that relationships between alcohol use and insomnia for women may differ depending on whether frequency of alcohol use or troublesome drinking are examined.

A large, longitudinal study of 9,941 Norwegian adults (53.6% were women) found that men reporting high levels of alcohol consumption at baseline were at higher risk of reporting sleeplessness at a follow-up 13 years later.[47] Similarly, men who experienced sleeplessness at baseline also were at higher risk of reporting high levels of alcohol consumption at the follow-up, demonstrating the bidirectionality of associations between sleep problems and alcohol use. In contrast, no such relationships were found for women.

A population-based study of 3,450 French adults (52.4% were women ages 18 to 64) reported that drug use for insomnia (prescription or nonprescription) was associated with alcohol misuse among men but not among women.[48] The only study of insomnia prevalence among individuals in treatment for AUD found that women and men reported similar rates of insomnia symptoms, despite a larger prevalence of insomnia among women in the general population.[49] Also, insomnia symptoms at baseline were significantly associated with relapse to AUD for both men and women.

The extant data are mixed regarding whether women show differential risk for associations between self-reported sleep disturbance and alcohol use. However, these observational studies, which rely entirely on self-report methods to measure both alcohol use and sleep disturbance, use different questionnaires and, in some cases, use measures limited to a single item. More research is needed to characterize the relationship between sleep behavior and alcohol use among women, especially studies that help distinguish sleep problems as predictors of relapse and alcohol use as a predictor of insomnia. Further investigation should use more comprehensive, frequent measures of sleep behavior (e.g., sleep diaries) potentially combined with objective measures (e.g., actigraphy) and measures of alcohol consumption to better characterize sex differences in these relationships.

Sleep as a Predictor of Adolescent Alcohol Use

As early as childhood, self-reported sleep problems are related to onset of substance use in adolescence.[50] In the first prospective study of sex differences in this relationship, Wong and colleagues found that sleep problems in childhood were a significant predictor of onset of drinking in both boys and girls but at earlier ages for boys (8 to 14) than girls (15 to 17).[51] In a large, community-based sample of 7,507 children and adolescents in Hong Kong (48.5% were females ages 6 to 17), Zhang and colleagues found that boys with insomnia symptoms were more likely to report regular consumption of alcohol (sometimes or often), whereas no such relationship was found for girls.[52]

In a population-based study of 4,187 Finnish adolescents (51.8% were females ages 11 to 15), perceived tiredness was related to increased likelihood of drinking and smoking for boys, but for girls it was only related to an increased likelihood of smoking.[53] In contrast, in a large sample of 13,381 U.S. adolescents (48.8% were females ages 12 to 17), there was a stronger relationship between subjective sleep problems and substance use in general (i.e., use of cigarettes, alcohol, or illicit drugs) for girls than for boys.[54]

Unpublished data from Hasler and colleagues (2017) suggest that in a sample of 729 adolescents (368 were females ages 12 to 21) from the National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) study, females with worse sleep quality were more likely to report binge alcohol use at baseline. However, males with worse sleep quality at baseline were at a greater risk of worsening binge alcohol use a year later.

Emerging data from longitudinal studies that track sleep patterns in adolescents before the onset of alcohol use suggest there may be sex differences in the relationships between sleep behaviors and alcohol use.[50] However, further data are required before definitive conclusions can be reached. Such work is needed to determine sex differences in the directionality of the relationships between substance use and sleep and circadian factors, as well as the underlying mechanisms of these relationships.

Sleep and Circadian Timing

Circadian rhythm disturbance can underlie sleep problems, and alcohol use alters many circadian functions (e.g., blood pressure, body temperature, hormone release).[55] Proper assessments of melatonin level, cortisol level, or body temperature, which are validated methods for measuring circadian rhythm, require rigorous laboratory protocols conducted over multiple hours to days and, thus, are not always feasible. Measurements of circadian preference (i.e., morningness-eveningness), chronotype, or sleep timing can serve as proxies for direct measures of circadian patterns of sleep–wake activity. To our knowledge, no studies have directly examined whether sex moderates the relationship between alcohol use and circadian rhythms in humans. One preclinical study that used mice with a knockout of adenosine equilibrative nucleotide transporter type 1 (ENT1), which is associated with both AUD and circadian/sleep disruptions, showed that circadian rhythm disruption increased alcohol consumption in male but not female mice,[56] suggesting that further investigation of sex differences in this area is warranted in humans.

Although more bona fide circadian research is needed, proxies for circadian rhythm, such as eveningness and late chronotype, consistently are associated with more alcohol use and problems with alcohol.[57] On average, women tend towards a relatively earlier sleep and activity pattern (i.e., morningness/early chronotype), which theoretically might lower the risk of alcohol use associated with circadian factors.

Hasler and colleagues investigated the effect of sleep timing on response to alcohol among 148 young adults (50 were women ages 21 to 35).[58] In males (White males only) but not in females, later sleep timing and greater eveningness preference were associated with a greater self-reported stimulating effect of alcohol immediately following alcohol consumption. In addition, greater variability in sleep duration was related to

greater sedation following alcohol consumption for both men and women. Further work is needed to examine links between circadian factors and heavy alcohol use, particularly among adolescents, to establish potential sex-specific predictors of alcohol use.

CLINICAL CONSIDERATIONS AND TREATMENT

Some sleep abnormalities may predate the effects of alcohol and also may differ between men and women. In addition, the prevalence of different sleep disorders must be taken into consideration. As already described, women are 40% more likely to develop insomnia than men.[20] Individuals may be vulnerable to the development of insomnia for a variety of reasons.[1] Predisposing factors such as genetics (e.g., *CLOCK* gene polymorphism or family history of AUD), childhood trauma, and childhood sleep problems increase an individual's risk of developing insomnia. Precipitating factors are stress-promoting events that trigger acute insomnia. Perpetuating factors are maladaptive compensatory behaviors, such as reading in bed or drinking alcohol, used to cope with sleep difficulty. Screening women for sleep problems may help providers intervene before problematic use of alcohol develops or may increase the likelihood of maintaining abstinence.

Pathways toward alcohol use vary developmentally, and sleep characteristics during childhood and adolescence predict risk for onset of alcohol use and misuse.[59] Childhood sleep problems are related to the onset of alcohol use in adolescence; therefore, treating sleep problems early in life may confer some benefit by delaying the onset of alcohol use. Furthermore, sleep disorders often manifest during reproductive transitions (e.g., puberty, pregnancy, menopause).

Females tend to develop insomnia after puberty, and the later sleep timing that occurs during puberty is positively associated with alcohol use.[16] Addressing the sleep disturbances of pregnant women is especially important. Alcohol consumption during pregnancy acutely affects fetal sleep behavior, and research suggests that prenatal alcohol exposure is related to persistent sleep disruption in affected children.[60] For many women, sleep disturbance and complaints of insomnia increase during and after the menopause transition.[12] The sleep changes related to aging, hormonal fluctuations, and psychological adjustment may contribute to women in this age group being particularly vulnerable to developing AUD.[61]

Improved understanding of the mechanisms by which these hormones modulate sleep may help guide development of novel therapies for treatment of problematic alcohol use. Such studies will help health care providers make informed decisions about medications (and dosages) and behavioral interventions that will be effective for treating sleep problems among women with AUD.

Cognitive behavioral therapy for insomnia is the first line of treatment for insomnia and is equally effective for men and women.[8,62] This nonpharmacological treatment method focuses on behaviors, cognitions, and associations that contribute to poor sleep.[63] The therapy uses a combination of sleep restriction (i.e., limiting time spent awake in bed), stimulus control, sleep hygiene (that is, healthy sleep habits such as consistent bed and wake times, comfortable bedroom environment, or avoiding caffeine and alcohol before bedtime), and cognitive therapy to address distorted beliefs about sleep. Up to 80% of patients benefit from this therapy, and treatment effects are maintained at follow-up a year later.[9] Pharmacotherapy is the next evidence-based approach for treatment of sleep disturbance, and it often is used in conjunction with cognitive behavioral therapy for insomnia, although it can be contraindicated for individuals with AUD.

Although women tend to have better long-term treatment outcomes than men, they are less likely to receive services specifically for alcohol-related issues, and they are more likely to seek treatment in settings that are not alcohol specific.[39] Educating health care providers in the primary

care setting to screen women for AUD and sleep problems may help reduce the stigma many women face when seeking appropriate treatment for AUD.

In addition, management of sleep problems is not typically a first line of treatment for individuals with AUD, despite the association between insomnia symptoms and increased risk of relapse. Sleep is a modifiable behavior that, if improved, may have downstream benefits for other health outcomes.[23] Medication trials (e.g., trazodone, gabapentin, quetiapine) have shown mixed efficacy and can be contraindicated in individuals with AUD, whereas behavioral treatments for insomnia consistently have been more effective in treating sleep problems, with moderate to large effect sizes.[1]

Treating sleep problems early may reduce risk for subsequent AUD. Considering that for women depressive symptoms predict alcohol consumption, cognitive behavioral therapy for both insomnia and depression may help prevent problematic alcohol use with two points of intervention. Although cognitive behavioral therapy for insomnia has not been shown to differentially improve alcohol outcomes,[64,65] more randomized controlled trials are warranted. This therapy has already shown promise as a treatment for insomnia among individuals with AUD, and men and women with no AUD respond to the therapy equally well.[66] It will be valuable for future studies to investigate the utility of cognitive behavioral therapy for insomnia and of other treatments that aim to improve sleep in individuals with AUD, as well as to examine whether these treatments are equally effective in men and women.

FUTURE DIRECTIONS AND CONCLUSION

Suggested areas for future research on sex differences related to alcohol and sleep include examination of:

- Alcohol's neurotoxic effects on circuits important for sleep generation
- Sleep during sustained abstinence from alcohol
- Cardiovascular functioning at night following alcohol use
- Alcohol use and its relationships with circadian misalignment and shiftwork
- Hormonal change and reproductive phase (e.g., puberty, the menstrual cycle, pregnancy, menopause) effects on alcohol use and sleep
- Other demographic factors (e.g., age, race, ethnicity, socioeconomic status) and how they affect alcohol use and sleep
- Longitudinal studies of sleep before initiation of alcohol use and across the course of recovery in individuals with AUD who are abstinent
- Cognitive behavioral therapy for insomnia and other treatment efficacy and effectiveness in improving sleep for individuals with AUD

Women historically have been underrepresented in research studies on alcohol use and sleep. Although AUD currently is more prevalent among men, the male/female differences in patterns of alcohol consumption are converging. Now, more than ever, sex differences need to be considered in all aspects of alcohol research. Only a small body of literature has investigated sex differences or interactions with sex in relation to sleep outcomes and alcohol use, making it challenging to draw definitive conclusions from the research thus far. Sleep and alcohol use vary by race and ethnicity,[67] and further research examining these characteristics in the context of sex differences is needed.

In addition to understanding sex differences in the relationship between alcohol and sleep, understanding the consistencies in the effects of alcohol on sleep among men and women is important. Alcohol has the same detrimental effects on many aspects of sleep and sleep physiology, regardless of sex. Given that sleep disturbance is so commonly reported by individuals with AUD, and the strong associations among sleep, daytime functioning, and mental and physical health, understanding how these relationships might differ in women compared to men is crucial to developing targeted and appropriate treatment recommendations.

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Co-Occurring Alcohol Use Disorder and Anxiety

Bridging Psychiatric, Psychological, and Neurobiological Perspectives

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A substantial number of people who have problems with alcohol also experience strong anxiety and mood problems. This article provides an overview of the evolving perspectives of this association in the context of three related disciplines—psychiatry, psychology, and neuroscience. Psychiatric and epidemiological studies show that having either an anxiety- or alcohol-related diagnosis elevates the prospective risk for developing the other disorder. From the psychological perspective, behavioral research demonstrates that drinking to cope with negative affect is a potent marker for current and future problems with alcohol. Neuroscientific research implicates overlapping neurobiological systems and psychological processes in promoting the rise of negative affect and alcohol misuse. The psychiatric perspective that alcohol misuse and co-occurring anxiety represent neurobiologically distinct diagnostic conditions has dominated the field for many decades. However, recent research provides increasing support for the neuroscientific perspective that these conditions share underlying, mutually exacerbating, neurobiological processes.

KEY WORDS: alcohol; anxiety; comorbidity; negative affect; stress

Introduction

"Those who cannot remember the past are condemned to repeat it."
—*George Santayana*

Few observations in psychiatry have been documented as long and as consistently as the association between anxiety (and general negative affect) and the chronic misuse of alcohol. Research has shown that up to 50% of individuals receiving treatment for problematic alcohol use also met diagnostic criteria for one or more anxiety disorders.[1,2] This percentage can be compared with the prevalence of current (within the past 12 months) anxiety disorders in the U.S. community, which is estimated to be 11%.[3,4]

The psychiatric, psychological, and neuroscientific disciplines have developed theories to explain the association between alcohol and anxiety disorders. Each discipline has independently contributed to the understanding of how to best describe and treat alcohol use disorder (AUD) in the context of negative affectivity. However, very little cross-communication has occurred among these disciplines. This insularity and particularism continue to impose significant opportunity costs in this field.

A key challenge to applying a comparative perspective across disciplines and time is the use of unique and evolving terminology and definitions for similar phenomena. Terms such as anxiety, anxiety disorder, depression, mood disorder, tension, stress, stress disorder, and negative affect are used differently across disciplines and time. The relationships among these constructs can be conceptualized as a Venn diagram, with the shared spaces representing overlapping constructs. In these overlapping spaces, the greatest opportunities for integration across disciplines can be found. In this review, the term "negative affect" (i.e., negative hedonic tone and the biology that underpins it) describes the shared psychological and biological space for related constructs of anxiety, tension, stress-responding, and anxiety disorder.

First, historical trends and research related to the psychiatric classifications of alcohol misuse, negative affect, and their co-occurrence are reviewed, including typologies and diagnoses. Next, a history of behavioral examinations of negative affect and alcohol misuse is presented from the psychological perspective, along with a discussion of research on the use of alcohol to cope with negative affect. Finally, neurobiological research on the relationship between negative affect and alcohol use is reviewed, and the opponent process model is explained. The concluding section synthesizes the discipline-specific research to identify conclusions and unanswered questions about the connections between alcohol use and negative affect.

Psychiatric Disorder Classifications and Diagnoses

Typologies are the oldest formal approach to categorizing alcohol misuse accompanied by strong negative affect. Summarizing dozens of such typologies from the past 200 years, Babor observed that virtually all identified an anxious-depressed subtype (Apollonian) and a revelry-oriented, rule-breaking subtype (Dionysian).[5] The promulgation of these typologies occurred primarily in the "prescientific" era (before the 1940s), but their legacy remains evident today.

For example, Cloninger described a model in which heritable personality traits set the stage for the development of Type I or Type II "alcoholism."[6,7] Type I included people whose problems with alcohol use began later in adult life, often contemporaneous with increasing negative affect or stressful life experiences. These individuals were characterized as shy, anxious, and pessimistic (Apollonian), and their alcohol use was believed to be motivated by an effort to cope with the unpleasant subjective experiences associated with these traits. Type II included people whose problems with alcohol use began early in adult life, without reference to environmental conditions or fluctuations in internal emotional states. These individuals were characterized as having relatively less fear and guilt while engaging in relatively more rule-breaking and antisocial behavior (Dionysian), often including drinking alcohol and other drug use. Past and present typology approaches share the view that negative affect is not a separate, co-occurring condition but rather an inherent trait of a significant subtype of people who have problems with alcohol.

Comorbidity paradigm

By the middle of the 20th century, medically oriented researchers increasingly attempted to categorize and quantify psychopathological and medical conditions observed among people being treated for the chronic misuse of alcohol.[8] Unlike earlier typologies in which strong negative affect was considered an inherent trait of a subtype of people who had problems with alcohol, this descriptive, medical approach viewed strong anxiety and other psychiatric problems as distinct, diagnosable conditions that often co-occur with alcohol-related conditions. This conceptualization led to co-opting the medical term "comorbidity" to indicate the presence of two or more distinct psychiatric disorders.[9] The psychiatric paradigm of comorbidity was first fully realized and codified nearly 40 years ago in the third edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM).[10] In

the more recent DSM-5, the paradigm remains the standard psychiatric model for describing, characterizing, and treating co-occurring negative affect and AUD.[11]

Epidemiology of co-occurring disorders

Within the co-occurring psychiatric disorder (comorbidity) paradigm, and armed with the DSM's observable and reliable diagnostic criteria, several large, epidemiological surveys have quantified the relative risk for an alcohol-related diagnosis in the presence versus absence of a diagnosed anxiety disorder. The largest and most comprehensive community-based surveys in the United States include the Epidemiologic Catchment Area study ($N \sim 20{,}000$), the National Comorbidity Survey ($N \sim 8{,}000$), and the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC, $N \sim 43{,}000$).

Alcohol-related diagnoses

An important issue in interpreting epidemiological findings is the diagnostic definition of AUD. The DSM-IV included two separate alcohol-related diagnoses: alcohol abuse and alcohol dependence.[12] A DSM-IV diagnosis of alcohol abuse required a maladaptive pattern of ongoing drinking resulting in multiple impairments. Some impairments that met the criteria were: not fulfilling major obligations at work, school, or home; using alcohol while driving or in other physically dangerous situations; having recurrent legal problems from driving under the influence, fighting, or other actions related to alcohol use; and experiencing exacerbation of interpersonal problems because of continued alcohol use.

A DSM-IV diagnosis of alcohol dependence required meeting at least three of seven criteria.[12] The first two criteria were physical—development of tolerance to alcohol and development of withdrawal symptoms. The remaining five criteria were behavioral signs of dependence, such as spending a great deal of time obtaining, drinking, or recovering from the effects of alcohol and drinking more alcohol, or for longer, than intended.

In the DSM-5, however, alcohol abuse and dependence have been integrated into a single diagnosis of AUD with mild, moderate, or severe subclassifications.[11] The separate classifications of alcohol abuse and alcohol dependence were removed.

Most available epidemiological studies used diagnostic criteria from DSM-IV or earlier, and they uniformly showed a positive association between anxiety or mood disorders and alcohol dependence but not alcohol abuse. A synthesis of the major epidemiological studies showed the risk (odds) for meeting diagnostic criteria for alcohol dependence more than doubled ($OR = 2.3$) among individuals with an anxiety disorder compared to those with no anxiety disorder.[13] However, the odds of receiving a diagnosis of alcohol abuse alone were about the same for individuals with or without an anxiety disorder ($OR \sim 1$). These results suggest that the association between anxiety disorders and AUD will diminish in forthcoming epidemiological findings (e.g., in results from the NESARC III) that use the DSM-5 diagnosis criteria.

Anxiety disorder diagnoses

Parallel to the question of how the definitions for alcohol-related diagnoses affect the magnitude of the association with anxiety disorders is the question of how the definitions for anxiety disorders affect that association. An early analysis[14] of research on co-occurring disorders in the 10 years following the introduction of DSM-III criteria reached the provisional conclusion that each major subtype of anxiety disorder (i.e., social phobia disorder, panic disorder, and generalized anxiety disorder)[10] had a unique relationship to alcohol misuse, presumably because of distinct neurobiology and symptom manifestations (e.g., discrete symptom triggers, omnipresent symptoms, or random symptom episodes). This conclusion fit neatly within the zeitgeist of that era, which presumed important clinical and biological distinctions for all psychiatric diagnoses.[10,13]

However, restricting attention to a single diagnosis and its relationship to alcohol misuse does not align with more recent research. For example, it is now better understood that various anxiety disorder subtypes are commonly present in the same individual.[15,16] Therefore, conclusions based on epidemiological findings that focused exclusively on one anxiety disorder diagnosis without accounting for the likely presence of additional anxiety subtypes have become suspect. Also, the conclusion that each

anxiety disorder subtype has a unique association with alcohol misuse is inconsistent with research showing that all the subtypes individually confer a similar increase in risk for alcohol misuse,[13] and that the risk increases substantially for each additional anxiety disorder subtype.

Recent "big data" modeling approaches have advanced the understanding of epidemiological data related to the association between anxiety disorder subtypes and risk for alcohol misuse. Seminal work using this approach comes from Krueger, who applied structural equation modeling of latent variables related to anxiety and depression diagnoses.[17] This research showed that a large proportion of the covariation in anxiety or mood disorder diagnoses could be characterized along a single continuum called "negative emotionality." However, some of the variance of specific anxiety disorders was distinct from the negative emotionality continuum; that is, some variance was unique to a specific anxiety disorder subtype.

Kushner and colleagues applied this analytic approach to NESARC data to assess the relationship between risk for alcohol misuse and the shared versus unique components of several anxiety and depressive disorders.[18] This analysis showed a strong positive relationship between risk for DSM-IV alcohol dependence and the shared components of the anxiety and depression diagnoses. However, the analysis also showed virtually no relationship between risk for alcohol dependence and the unique components of those diagnoses. These findings are inconsistent with the idea that each anxiety disorder has a unique association with the risk for alcohol misuse. Instead, the results suggest that all anxiety and mood disorders contribute to general negative emotionality, which, in turn, correlates with the risk for alcohol dependence.

Temporal and causal priority

The elevated risk for alcohol misuse in the presence of anxiety represents a positive correlation between these conditions. One of the co-occurring conditions could be causing the other, but a third, unmeasured factor could be causing an increased risk for both conditions. When medical conditions correlate, the search for causality commonly starts by evaluating which condition preceded the other. This approach is based on the logical truism that an effect cannot precede its cause. However, preceding conditions do not necessarily cause later outcomes—the logical fallacy called "post hoc, ergo propter hoc." Still, studies have sought to illuminate the causal associations between the co-occurring disorders by determining which began first.[19] This research has shown that the onset of anxiety disorders preceded alcohol misuse in up to three-quarters of the people who had both conditions,[14] especially for those who had social anxiety disorder.[20]

Failing to clearly distinguish between temporal priority and causal priority is common in interpretation of order-of-onset studies.[20,21] Since its third edition, the DSM's hierarchical diagnostic scheme designates anxiety disorders in the presence of alcohol disorders as an alcohol-induced condition unless the anxiety symptoms presented first or persisted during a period of protracted abstinence.[11,12] This approach not only risks the logical error already discussed but also risks conflating initiating factors with maintaining factors. That is, this approach ignores the possibility that alcohol misuse played some role in the initiation of anxiety symptoms that over time evolved into independent anxiety disorders. However, these logical concerns may be moot empirically, because NESARC data show that the prevalence of substance-induced anxiety and mood disorders among individuals with a diagnosed alcohol disorder is vanishingly small.[4] Unfortunately, clinical guidelines designed to avoid mistaking substance-induced anxiety or mood problems for other anxiety or depressive disorders discourage clinicians from providing effective treatments for these conditions in people who are actively drinking or recently abstinent.[22]

Prospective relative risk

Compared to retrospective assessments of the order of onset for co-occurring disorders, assessments of prospective relative risk (i.e., the risk for developing a condition given the presence or absence of another condition) provide more information about conferred risk. For example, people typically experience onset of social anxiety disorder before they are old enough to legally purchase alcohol, so the anxiety disorder typically precedes problems with alcohol. Therefore, retrospective assessments showing that social anxiety disorder commonly

precedes problems with alcohol superficially suggest that the former causes the latter. However, this type of examination provides no information about the effects of alcohol misuse on later development of social anxiety disorder.

Prospective relative risk avoids problems related to retrospectively examining the order of onset. In a study by Kushner and colleagues, the prospective relative risk of alcohol dependence and several common anxiety diagnoses was examined among approximately 500 college students during their first year, senior year, and third postgraduation year.[21] Although anxiety disorders were more common than alcohol dependence at all assessment years, the prospective risk for new onset of either condition in a later assessment was two to five times greater if the other condition was present at an earlier assessment. Both conditions substantially increased the prospective relative risk for developing the other.

Effects of co-occurrence on alcohol treatment outcomes

Data show that individuals who have co-occurring anxiety or depressive disorders and alcohol-related disorders have a poor response to treatment for alcohol misuse.[23,24] For example, Kushner and colleagues reported that more than twice as many participants who had alcohol-related disorders and co-occurring anxiety or mood disorders, versus participants with no anxiety or mood disorder, returned to any drinking within 4 months following intensive residential treatment for alcohol misuse (52% vs. 21%).[1]

Efforts to mitigate the deleterious effects of co-occurring anxiety disorders on alcohol treatment outcomes, as well as to illuminate causal influences between these conditions, have inspired investigations into how treatment for one co-occurring condition affects symptoms of the other condition. For example, if an anxiety disorder maintains alcohol misuse, effectively treating the anxiety should reduce alcohol use and reduce the likelihood of relapse after treatment. In one study, researchers administered paroxetine or placebo in a double-blind fashion to participants who had AUD and social anxiety disorder.[25] They found that although the medication was clinically effective in reducing social anxiety symptoms, alcohol use severity was unchanged.

Several clinical trials have examined the effect of supplementing standard AUD treatment with a validated treatment for anxiety or mood disorders among individuals with both conditions. A meta-analysis of 15 randomized controlled trials, in which medication or cognitive behavioral therapy for co-occurring anxiety or depressive disorder was added to standard treatment for AUD, showed results similar to the paroxetine study.[25,26] That is, the meta-analysis showed that conventional treatments were effective at reducing co-occurring symptoms of anxiety and depression, but they did not meaningfully improve alcohol-related treatment outcomes.

Psychological Theories

In parallel to the evolution of the descriptive psychiatric paradigm for co-occurring disorders, early psychological researchers began studying alcohol's tension-reducing properties in laboratory (typically animal) models.[27] It is often forgotten (or at least ignored) that this early experimental work began as a test of Freud's theory that alcohol misuse served as an externalized ego defense mechanism. However, the research soon developed into operant-behavioral examination of what was called the "tension-reduction hypothesis." The hypothesis maintained that alcohol's pharmacological properties reduced tension, and this effect resulted in escalated drinking through negative reinforcement (i.e., reward generated by diminution of a noxious stimulus). In this research, the tension was any noxious state (e.g., frustration, approach-avoidance conflicts, or pain) that elicited a subjective or physiological stress response. Many dozens of laboratory studies through the latter half of the 20th century tested the tension-reduction hypothesis. Ultimately, however, the cumulative results were deemed to be "negative, equivocal, and contradictory."[28]

In reaction to the early experimental failures and ambiguities of the operant-behavioral tension-reduction hypothesis, psychological researchers increasingly deemphasized alcohol's putative pharmacological effects on tension. They began to emphasize the subjective expectancies, beliefs, and motivations presumed to affect a person's decision to drink when experiencing negative affect.[29] Drinking to cope with negative affect was viewed

as a primary drinking motive.[30] Keeping with the tension-reduction hypothesis, these researchers did not focus on formal diagnostic categories for negative affect or alcohol misuse.[31] However, other research has linked drinking-to-cope motives with individuals who met diagnostic criteria for co-occurring AUD and anxiety disorder.[19]

An analysis of NESARC data has demonstrated that individuals who reported using alcohol to cope with the symptoms of anxiety disorder are at increased risk for persistent alcohol dependence.[19,32] In addition, people with anxiety disorders who reported drinking to cope had a fivefold increased risk for developing alcohol dependence within 3 years.[32] People with anxiety disorders who did *not* drink to cope had virtually the same prospective risk for developing alcohol dependence as people with no anxiety disorders. Further, people with anxiety disorders who did not report any drinking to cope drank less daily than people with no anxiety disorder.

Neurobiological Theories

Starting in the 1970s, the increasing availability of biological measures offered researchers an opportunity to study the effects of alcohol on stress-responding (and vice versa) in more refined and controlled ways. This allowed for distinctions between subjective (e.g., self-reported) and objective (e.g., serum cortisol) responses to stress, as well as between immediate stress reactivity and subsequent stress regulation. Surprisingly, distinguishing subjective and objective stress-response measures revealed little connection between the two, with the former relating more directly to predictions from the tension-reduction hypothesis.[33] Early research on stress and alcohol used these technological advancements to test the operant tension-reduction hypothesis, albeit with mixed results.[34]

Psychophysiological and neurobiological correlates

Beginning in the 1990s, stress-related alcohol research evolved from its roots in tension-reduction research to become a multifaceted subspecialty focused primarily on the psychophysiological and neurobiological correlates of the stress response, stress regulation, and alcohol misuse. Increasingly, this research includes examination of the long-term genetic and environmental influences on stress reactivity and regulation and their connections to the development of AUD vulnerability.

For example, Brady and Back reviewed research linking early trauma and exposure to chronic stressors with permanent dysregulation in the brain systems implicated in the pathophysiology of depression, anxiety, and addiction.[35] Other investigators reviewed research that reported associations between alcohol dependence or genetic risk for alcohol dependence and dysregulated patterns of laboratory stress-responding.[36,37] Several studies have implicated chronic alcohol misuse in the dysregulation of the stress response, which contributed to further alcohol craving and increased likelihood of relapse.[38-40] These and related studies demonstrate that heritable traits associated with risk for alcohol-related disorders; as well as environmental insults such as acute trauma, chronic stress, and chronic alcohol misuse; can produce durable neurobiological and subjective stress-response changes that have been associated with the development or persistence of both AUD and anxiety disorders.

Opponent process model

Koob and colleagues have placed both the neurobiological and subjective experiences of stress-responding and negative affect at the very center of addiction pathology (Figure 1).[41] More specifically, they conceptualized addiction as a three-stage, pathodevelopmental cycle that engages executive function, incentive salience, and negative emotionality at different degrees during specific stages of addiction. In this opponent process model, the term "addiction" refers to the neurobiological and motivational changes that occur as a consequence of chronic substance use.

The first stage—binge/intoxication—involves activating reward circuits (e.g., the release of dopamine and opioid peptides in the ventral striatum) in response to alcohol or other drug use, which also engages incentive salience circuits.[41] In this early stage of addiction, positive reinforcement from direct activation of the brain's positive valence systems, as well as from formerly neutral stimuli that have become classically conditioned to evoke a pleasurable response, motivates ongoing and

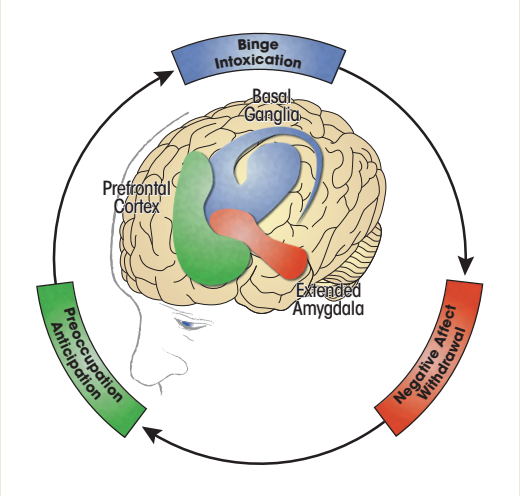

Figure 1 Addiction cycle stages and associated brain regions. *Source:* Adapted from U.S. Department of Health and Human Services, Office of the Surgeon General. *Facing Addiction in America: The Surgeon General's Report on Alcohol, Drugs, and Health*. Washington, DC: U.S. Department of Health and Human Services; November 2016.

increased substance use. This is characterized as the impulsive stage of addiction because the goal of increasing pleasure, rather than avoiding or escaping discomfort, motivates seeking alcohol or other drugs.

In response to chronic alcohol or other drug use, both within-system and between-system brain processes seek homeostasis through dynamic, neuroregulatory, countervailing effects.[41] However, as chronic use continues, homeostasis gives way to neuroadaptations that reset the baseline operation (allostasis) in these systems. These allostatic adaptations in the brain lead to the second stage of addiction—withdrawal/negative affect. In this stage, reward circuits become blunted because of within-system neuroadaptations. The brain's stress systems, including corticotropin releasing factor and norepinephrine in the central amygdala and bed nucleus of the stria terminalis, become increasingly dysregulated because of between-system compensatory neuroadaptations. At this point in the addiction process, subjective negative affect predominates, especially during periods of sobriety and withdrawal. This later stage of addiction marks a shift from impulsive use driven by positive reinforcement to compulsive use driven by negative reinforcement. In this stage, compulsive substance use is aimed, in part, at decreasing the negative affect caused or aggravated by the allostatic reset in the brain's stress and mood systems.

Finally, after these neuroadaptations have been established, the third stage of addiction—preoccupation/anticipation—undermines attempts at abstinence from drinking.[41] At this point, chronic alcohol or other drug use becomes an integral, exogenous input for maintaining equilibrium in the brain's mood and stress regulation systems.

Preclinical research supports the tenets of the neurobiological opponent process model.[42] Although the model has not yet been translated to validated clinical applications, it informed the development of the Addictions Neuroclinical Assessment, a framework that uses neuropsychological data that correspond to the three stages of the neurobiological opponent process model to classify the individual differences in AUD to improve diagnosis and treatment.[43] The model does imply specific treatment targets, such as corticotropin releasing factor[44,45] and alpha$_1$-noradrenergic systems.[46] Simpson and colleagues found clinical benefit from prazosin, an alpha$_1$ antagonist, in participants with an alcohol dependence diagnosis.[47] However, the only study to examine prazosin in a sample of people with co-occurring disorders (alcohol dependence and post-traumatic stress disorder) reported that the medication had no effect on stress-responding or alcohol treatment outcomes.[48]

The opponent process model also implies that psychosocial treatments could usefully target the motive of using alcohol to cope with negative affect. Epidemiological data and the opponent process model both support the concept that this motive is a primary link between the neurobiological and subjective manifestations of negative affect and drinking behavior.[49]

Discussion and Future Directions

The term "comorbidity" has become a fairly generic reference for co-occurring alcohol and anxiety or depressive disorders. Yet ontologically, the presence of two or more distinct, clinical diagnoses remains firmly fixed in an increasingly strained medical-diagnostic paradigm of psychopathology

classification. Central to this strain is the assumption that specific diagnostic dyads are the appropriate unit of analysis for studying co-occurring negative affect and alcohol misuse. However, negative affect is common to many anxiety and depressive disorders and can increase the risk for alcohol misuse, particularly when drinking to cope with negative affect is the motive.

Unidirectional causation theories

The notion of a simple, unidirectional, causal link between co-occurring disorders is not supported by the findings reviewed in this article. A prospective study has shown that either experiencing clinical-level anxiety or engaging in chronic alcohol misuse increases the risk of developing the other.[21] In addition, clinical research shows that effectively treating one co-occurring condition does not substantively affect the other. Viable explanations for the relationship between co-occurring conditions include the possibility of a common cause for both conditions or bidirectional causation between the conditions. For example, dysregulated stress response or regulation may be a common risk factor for the development of both alcohol and anxiety disorders.

Also, the concept of causation among co-occurring conditions may be based on an incorrect assumption. Rather than two distinct conditions, each requiring a cause, negative affect and alcohol misuse may be parts of a single, neurobiological-behavioral syndrome. This view aligns mostly with recent neurobiological theories of addiction, but it also shares similarities with early typologies, in which negative affect was considered a fundamental trait among a large subgroup of people who had problems with alcohol.

Shared neurobiology

The research reviewed in this article shows that trauma and chronic stress, as well as a familial risk for problems with alcohol, are associated with the dysregulated stress-response systems implicated in the development of both alcohol and anxiety disorders. In addition, chronic alcohol use is associated with dysregulated stress-responding, which, in turn, is associated with relapse following treatment for alcohol problems. Collectively, these and related findings point to overlapping neurobiological vulnerabilities.

The overlapping neurobiology of negative affect and AUD is supported by several lines of research that implicate specific brain circuits related to both conditions. The central amygdala regulates negative affect states,[45,50] and research suggests the central amygdala plays a role in physiological and behavioral responses to stress, anxiety, and alcohol- or drug-related stimuli. Similarly, human imaging and animal research demonstrate abnormal central amygdala function in individuals with alcohol or anxiety disorders.[50] A consensus is building that the central amygdala serves as a central hub for anxiety and alcohol circuits owing to its strong connection and influence on brain areas involved in executive function (medial prefrontal cortex), emotion regulation, stress responsivity (paraventricular hypothalamus and locus coeruleus), and reward processing (nucleus accumbens shell and ventral tegmental area).[45,50-53] Crucial to the overlapping neurobiology conjecture, research shows that chronic alcohol use results in neuroadaptations to the central amygdala that are similar to the neuroadaptations that occur after chronic stress.[53] If the neurodysregulations underlying anxiety or mood conditions and alcohol misuse overlap, it becomes reasonable to hypothesize that the common co-occurrence of these conditions may be an outgrowth of this shared neurobiology.[54]

The shared neurobiology thesis implies several unique and nonobvious hypotheses. For example, having either condition should be a risk marker for developing the other. This is consistent with prospective, observational studies showing that having either an anxiety disorder or AUD at any time increases the relative risk for future development of the other disorder. The shared neurobiology view also implies that the transition from nonproblematic alcohol use to AUD (roughly corresponding to the withdrawal/negative affect stage of addiction in the opponent process model)[41] should require less overall alcohol exposure for people with anxiety and depressive disorders.

This hypothesis, called "telescoping," theorizes that having either condition indicates perturbed neurobiology that is also relevant to developing the other condition. Examinations of transitions from nonproblematic or no use to problematic use of alcohol or nicotine support the telescoping hypothesis.[55,56] People with anxiety disorders transitioned significantly faster than those with

no anxiety disorder from initial use milestones to substance dependence. This effect was more pronounced for people who had multiple anxiety or mood disorders, even after controlling for lifetime drug exposure.[57,58]

Anxiety problems in the absence of alcohol misuse

As already discussed, an analysis of epidemiological data shows that people who report drinking to cope with anxiety symptoms have increased prospective risk for developing alcohol dependence.[19,32] People with anxiety disorders who do not drink to cope with their symptoms do not have an increased risk for AUD. This is good news, because most people with anxiety disorders do not report drinking to cope with their symptoms, but it also raises questions. For example, why do some people with anxiety problems drink to cope and others do not? Also, if this population has no increased risk for AUD, how is that consistent with the shared neurobiology thesis? Perhaps currently unknown factors—cultural, psychological, or biological—protect these biologically vulnerable individuals by discouraging drinking to cope.

Alcohol misuse in the absence of anxiety

Not all people struggling with alcohol problems meet diagnostic criteria for anxiety disorders. As already discussed, an analysis of epidemiological data suggests that a DSM-IV diagnosis of alcohol abuse (i.e., negative consequences from alcohol use) without alcohol dependence does not correlate with anxiety disorder diagnoses.[13] The opponent process model suggests that all advanced cases of substance use disorder ultimately involve negative affect (although they may not necessarily manifest as diagnosable anxiety disorders), whereas the typology and medical/diagnostic models suggest that only a particular subgroup of people who have problems with alcohol will have the key feature of negative affect.

These different models are not necessarily irreconcilable when considering the patho-developmental trajectory of addiction. During the early binge/intoxication (impulsive) stage of addiction, the opponent process model would anticipate low levels of negative affect, but during the later stage of negative affect/withdrawal, the model specifies the presence of significant negative affect and drinking to cope. Cross-sectional snapshots of people who have significant alcohol problems might reveal groups with anxiety (Apollonian) and groups without anxiety (Dionysian), but, ultimately, all may become Apollonian types as addiction advances. People who manifest anxiety problems before alcohol problems may transition very rapidly (telescope) from binge/intoxication (Dionysian) to negative affect/withdrawal (Apollonian), whereas others may make this transition more slowly or, perhaps, never.

Stress reactivity and regulation

Stress responses in terms of both reactivity and regulation include frequently disjunctive, subjective and objective indicators. Curiously, subjective indicators of acute stress response commonly are elevated in individuals who have anxiety or alcohol problems, whereas the objective indicators tend to be acutely blunted, with diminished regulation.[58,59] Also, research has well-established that perturbations in the neurobiological systems that govern biological responses to stress are associated with poorer alcohol and other substance use disorder treatment outcomes.[38,53]

For investigators seeking to bridge the multiple disciplines included in this review, the findings concerning stress responses pose challenges and opportunities for future research. For example, can individuals with AUD be distinguished meaningfully based on objective stress reactivity and regulation indicators, and do subjective anxiety symptoms mark or moderate this distinction? For augmenting treatment for AUD, would targeting biological stress reactivity (e.g., hypothalamic pituitary adrenal activation) be more promising than targeting anxiety disorders? Among people who have problems with alcohol, do those with versus those without co-occurring anxiety disorder react differently to protracted abstinence and withdrawal in terms of severity and persistence of dysregulation of the stress response? Prospective studies across the distinct stages of treatment and recovery for alcohol-related disorders may shed needed light on the relationships between alcohol, anxiety, and stress reactivity and regulation. Such studies have the potential to reveal the trajectory of re-regulation of the stress response during abstinence and how

it relates to anxiety symptoms and relapse risk. Understanding these parameters could make a valuable contribution toward using the stress system as a recovery biomarker.

Limitations

This review of literature from multiple disciplines required sacrificing depth for breadth. The material cited is largely limited to seminal studies and other reviews. In addition, complex research on stress and neurobiology is discussed in ways sufficient to make particular points but without providing a comprehensive or in-depth description of the underlying work. Doing so is beyond the scope of this article, but the approach presented in this article runs the risk of oversimplifying complex topics and obscuring relevant details. Also, this review does not address potentially important individual differences, such as sex.

Finally, the assumption that common areas of construct space exist across the disciplines of psychiatry, psychology, and neuroscience is open to debate. For example, medically oriented researchers might view subclinical negative affect as qualitatively rather than quantitatively distinct from diagnosed anxiety disorders. Similarly, it could be argued that dysregulated biological stress responses share little construct space with subjective negative affect and drinking to cope. However, as already noted, a dysregulated stress response is a known biological marker for the development of anxiety disorders and AUD, as well as for relapse.

Conclusion

This review broadens the psychiatric perspective on the association between diagnosable alcohol and anxiety disorders to include the psychological/learning and neuroscientific disciplines. Cross-referencing and reconciling (if not integrating) discipline-specific approaches may reveal opportunities for synergy.

The opponent process model offers a uniquely suitable framework for transdisciplinary cross-referencing and integration. This neurobiological model aligns with the Research Domain Criteria[60] framework's approach to characterizing psychopathology and, thereby, avoids being trapped by the diagnostic specificity that has failed to survive empirical scrutiny. In this model, the roles of motivation and reinforcement in fundamental learning processes, which were first explored in the operant-behavioral tension-reduction hypothesis, are integrated within a pathodevelopmental framework for substance misuse. The model also accommodates individual differences in neurosusceptibility to AUD within brain systems known to be affected by stress, anxiety, and depression. To better evaluate how negative affect is associated with alcohol misuse, the opponent process model expands the scope from a narrowly defined subset of individuals with co-occurring alcohol and anxiety disorder diagnoses to include the wider range of individuals who have advanced to the negative affect/withdrawal stage of addiction. Finally, the model provides promising and specific neurobiological (e.g., corticotropin releasing factor) and psychological (e.g., drinking to cope) targets for novel interventions.

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The authors declare that they have no competing financial interests.

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Biobehavioral Interactions Between Stress and Alcohol

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In this review, the effects of stress on alcohol drinking are discussed. The interactions between biological stress systems and alcohol drinking are examined, with a focus on the hypothalamic pituitary adrenal axis, corticotropin releasing factor, dynorphin, neuropeptide Y, and norepinephrine systems. Findings from animal models suggest that these biological stress systems may be useful targets for medications development for alcohol use disorder and co-occurring stress-related disorders in humans.

KEY WORDS: alcohol; animal models; stress

Behavioral Interactions Between Stress and Alcohol

Epidemiological studies of humans suggest that stress increases alcohol drinking. For example, findings from the 2001–2002 National Epidemiologic Survey on Alcohol and Related Conditions show that the number of past-year stressors is positively associated with prevalence of current drinking, current binge drinking, and alcohol use disorder (AUD) diagnosis.[1] However, as with most epidemiological human studies, the temporal and causal relationships between stress exposure and alcohol drinking are difficult to determine. Therefore, studies using animal models represent a useful complement for examining relationships between stress and alcohol drinking. Keyes and colleagues reviewed key epidemiological findings that show that stress exposure is associated with increased risk for AUD.[1]

Historically, studies using animal models to test the relationship between stress and alcohol drinking have focused on stress-induced reinstatement of alcohol-seeking as a model of stress-induced alcohol relapse in humans. In this procedure, animals are trained to self-administer alcohol in an operant task, that behavior is then extinguished (by omitting alcohol as reinforcement for lever pressing), after which exposure to a stressor (e.g., footshock) reinstates lever pressing for alcohol (i.e., alcohol-seeking).[2] In fact, stress has consistently been shown to reinstate seeking of a variety of drugs, including heroin, cocaine, and nicotine.[3]

A more limited body of literature shows that stress may increase alcohol consumption, but this effect depends heavily on a number of factors, including the stressor and the alcohol-drinking model used, as well as the species, sex, and age of the experimental animals.[4] Studies that show stress-induced escalation of alcohol drinking in rodents, with or without prior experience of alcohol drinking, are summarized in Table 1.[5-11] Stress also can synergize with exposure to high doses of alcohol to produce faster and more robust escalation of alcohol drinking in mice.[12] However, it is noteworthy that many stress procedures do not produce escalated alcohol drinking in rodents, and there is a paucity of animal models for studying stress-induced escalation of alcohol drinking and related behaviors (e.g., anxiety).[13,14]

On the other hand, chronic exposure to high doses of alcohol (which is an animal model of alcohol dependence) increases stress reactivity during withdrawal. For example, rats[15] and mice[16] exposed to chronic high-dose alcohol, followed by restraint stress during withdrawal, show higher levels of stress-induced anxiety-like behavior (in the elevated plus maze test) and suppression of social interaction, respectively, compared to their alcohol-naïve counterparts.

Table 1 Studies of Stress-Induced Escalation of Alcohol Drinking in Rodents

Procedure	Developmental Stage at Exposure	Stressor	Alcohol-Drinking Procedure
Stress → Alcohol Drinking			
In Rats	Adult	Repeated footshocks[5]	Two-bottle choice drinking
	Adolescent	Postweaning social isolation[6*]	Two-bottle choice drinking and operant self-administration
In Mice	Adult	Repeated social defeat[7]	Two-bottle choice drinking
	Adolescent	Postweaning social isolation[8]	Two-bottle choice drinking
Alcohol Drinking → Stress → Alcohol Drinking			
In Rats	Adult	Single exposure to soiled cat litter[9†]	Two-bottle choice drinking
	Adult	Single exposure to bobcat urine[10†‡]	Operant self-administration
In Mice	Adult	Repeated social defeat or forced swim[11]	Two-bottle choice drinking

*Stress increased alcohol drinking only in male rats.
†Stress increased alcohol drinking only in rats that were highly stress reactive.
‡Stress increased responding for quinine-adulterated alcohol (aversion-resistant responding) in rats that were highly stress reactive.

Data from animal models suggest that stress may not only trigger relapse to alcohol drinking but also increase subsequent alcohol drinking. Animal studies also show that exposure to high doses of alcohol increases stress reactivity. These studies suggest that stress exposure may facilitate development of AUD in humans, which may increase the likelihood of developing a stress-related disorder, further exacerbating AUD. The precise mechanisms through which this occurs are unclear, but dysregulation of brain stress signaling systems is likely to play a central role. Stress and chronic alcohol exposure alter the activity of brain stress systems, and dysregulation of these systems has demonstrable effects on alcohol drinking. The next section summarizes key findings from animal studies regarding the interaction between alcohol and brain stress systems.

Neurobiological Interactions Between Stress and Alcohol

Although alcohol often is consumed to alleviate stress,[1] alcohol may activate some brain stress systems and may be considered a stressor itself.[17] A body of literature shows that dysregulation of brain stress systems induced by stress or chronic high-dose alcohol exposure contributes to escalation of alcohol drinking or to alcohol-seeking relapse. This section summarizes key findings from research on several brain stress systems that likely mediate stress-alcohol interactions.

Hypothalamic pituitary adrenal axis

One of the main physiological responses to stress is activation of the hypothalamic pituitary adrenal (HPA) axis. This process begins with release of corticotropin releasing factor (CRF) from cells in the paraventricular nucleus of the hypothalamus, which leads to increased release of adrenocorticotropic hormone in the pituitary, which stimulates glucocorticoid (cortisol in humans and corticosterone in rodents) release in the adrenal gland. Therefore, HPA activation is generally considered to be "pro-stress," but the effects of HPA activity and corticosterone level on stress-related outcomes (e.g., anxiety-related behaviors) may depend on several factors. In animals, administration of corticosterone systemically or into the brain increases alcohol drinking,[18] and systemic glucocorticoid receptor blockade with mifepristone reduces alcohol drinking,[19] suggesting that glucocorticoid signaling modulates alcohol drinking. In addition, research has shown that infusion of mifepristone into the central amygdala attenuated stress-induced reinstatement of alcohol-seeking,[20] suggesting that glucocorticoids act on specific brain regions to modulate alcohol relapse-like behavior.

Interestingly, in a study that used a predator odor stress model, a blunted plasma corticosterone response in rats following predator odor exposure predicted high stress reactivity (avoidance of a stress-paired context).[21] Also, systemic corticosterone treatment before the stress exposure reduced the percentage of animals that were highly stress reactive (Avoiders) and reduced the magnitude of their stress reactivity (avoidance).[22] After stress, the Avoiders exhibited increased alcohol drinking, as compared to the Non-Avoiders,[10] which suggests that failure to mount a proper HPA response to traumatic stress predicts later escalation of alcohol drinking, which is similar to the notion that failure to mount a proper HPA response to traumatic stress predicts later post-traumatic stress disorder pathology[23] and poor treatment response[24,25] in humans.

Studies of rodents have demonstrated that acute alcohol exposure (experimenter-administered or self-administered) stimulates corticosterone release, mimicking a stressor.[26,27] In one study that used a model of chronic, high-dose alcohol exposure, alcohol-dependent rats, when compared with control rats, showed lower basal corticosterone levels during withdrawal and smaller increases in corticosterone following experimenter-administered or self-administered alcohol.[27] However, this effect may depend on factors such as the rodent species[28] and whether total or free amounts of glucocorticoids were measured.[29] This response is akin to the blunted corticosterone response shown in Avoider rats following exposure to traumatic stress.

In addition, a high basal corticosterone level in rats has been shown to protect against stress-induced and corticosterone injection–induced exacerbation of anxiety-like behavior.[30] Therefore, a blunted corticosterone response to alcohol or stress may be a common mechanism through which chronic, high-dose alcohol or traumatic stress increases alcohol drinking and stress-related disorders. However, Perusini and colleagues found that inhibition of corticosterone synthesis before stress blocked stress-enhanced fear conditioning.[31]

Studies of rats also have shown that glucocorticoid receptor levels in the brain were elevated following chronic alcohol exposure, and that mifepristone blockade of glucocorticoid receptors in these rats, systemically or within the central amygdala, reduced escalation of alcohol drinking.[32] Collectively, these findings suggest that HPA function and glucocorticoid receptor signaling in the brain, perhaps in specific brain regions, are important targets for medications development for AUD and co-occurring stress-related disorders.

CRF system

Aside from being a critical component of the neuroendocrine stress response, CRF signaling in extrahypothalamic brain regions is also a critical mediator of stress-alcohol interactions. For example, intraventricular infusions of a CRF receptor antagonist have been shown to attenuate stress-induced reinstatement of alcohol-seeking in rats,[33] and systemic blockade of CRF_1 receptors has produced similar effects.[34] Systemic CRF_1 receptor blockade also has been shown to reduce escalated alcohol drinking after exposure to stress induced by predator odor (in rats)[35] or by social defeat (in mice).[36] In studies of alcohol-dependent animals, intraventricular infusions of the CRF receptor antagonist D-Phe-CRF(12-41) reduced escalated alcohol drinking for both rats[37] and mice[38] during withdrawal. This effect is mediated, at least in part, by the central amygdala, as infusion of D-Phe-CRF(12-41) into the central amygdala also has been shown to reduce escalated alcohol drinking in alcohol-dependent rats during withdrawal.[39] CRF effects on escalated alcohol drinking appear to be mediated largely by the CRF_1 receptor. For example, researchers have reported that systemic CRF_1 receptor blockade reduced escalated alcohol drinking in mice[40] and rats[41] after chronic exposure to high doses of alcohol.

Collectively, these findings suggest that neural processes mediated by CRF–CRF_1 receptor signaling play an important role in escalation of alcohol drinking, and in alcohol-seeking relapse, induced by stress or by chronic, high-dose alcohol exposure. For more detailed discussions of this topic, please refer to reviews by Phillips and colleagues,[42] Spierling and Zorrilla,[43] and Pomrenze and colleagues.[44]

Dynorphin system

Stress generally increases brain dynorphin levels,[45] and dynorphin signaling via kappa-opioid receptors (KORs) mediates stress effects on behavior. For example, chronic stress (repeated forced-swim or repeated footshock stress) has been shown to

produce dysphoria-like behaviors in mice that can be attenuated by systemic KOR blockade or by gene deletion.[46] In one study, systemic administration of KOR antagonists reduced stress-induced escalation of alcohol drinking and alcohol-induced place preference in mice.[47] In another study, systemic KOR blockade attenuated reinstatement of alcohol-seeking in rats, which had been induced by yohimbine (an alpha$_2$-adrenergic receptor antagonist often used as a pharmacological stressor).[48]

These results are complemented by findings that dynorphin-KOR signaling in the brain is enhanced by chronic, high-dose alcohol exposure. For example, alcohol-dependent rats, relative to nondependent controls, have been shown to exhibit higher dynorphin levels and increased KOR function in the amygdala during withdrawal.[49] In the same study, KOR blockers, administered systemically or directly into the central amygdala, reduced escalated drinking in alcohol-dependent rats during withdrawal. Reviews by Anderson and Becker[50] and Karkhanis and colleagues[51] provide further discussion on the role of this system in stress-alcohol interactions.

Neuropeptide Y system

In contrast to the CRF and dynorphin systems, the neuropeptide Y system is generally thought to produce anti-stress effects. For example, following predator odor exposure, rats that exhibited high stress reactivity had lower neuropeptide Y levels in the brain, relative to rats that had lower stress reactivity.[52] In the same study, an infusion of neuropeptide Y into the brain an hour after stress exposure reduced the number of rats that subsequently exhibited high stress reactivity. In another study, neuropeptide Y infusion into the brain, followed by yohimbine-induced stress, attenuated reinstatement of alcohol-seeking.[53]

Compared to alcohol-naïve controls, alcohol-dependent rats have been shown to exhibit lower neuropeptide Y expression in several brain areas associated with negative affect and motivation, including amygdalar, cortical, and hypothalamic subregions.[54] These results suggest that chronic, alcohol-induced neuropeptide Y deficits in the brain may contribute to escalation of alcohol drinking and to negative affect during withdrawal. In other studies, an intracerebroventricular infusion of neuropeptide Y into the whole brain[55] or specifically into the central amygdala[56] reduced escalation of alcohol drinking in alcohol-dependent rats, suggesting that modulation of neuropeptide Y signaling in the brain may have therapeutic value in the treatment of AUD.

Both neuropeptide Y receptor subtypes (Y_1 and Y_2) have demonstrated roles in regulating alcohol drinking in rodents. For instance, intraventricular infusion of a Y_1 receptor agonist or a Y_2 receptor antagonist has been shown to reduce alcohol drinking in mice.[57] In a study of rats, the ability of a Y_2 receptor antagonist (via intracerebroventricular administration) to reduce alcohol drinking may have been potentiated in animals that were chronically exposed to high-dose alcohol.[58] However, Kallupi and colleagues found that a Y_2 receptor antagonist (administered systemically or into the central amygdala) attenuated only anxiety-like behavior, but not alcohol drinking, in rats chronically exposed to high-dose alcohol.[59]

Researchers have reported that Y_1 and Y_2 receptors regulate alcohol drinking in a brain region–specific manner. For example, research has demonstrated that Y_1 receptor activation or Y_2 receptor blockade in the medial prefrontal cortex reduced alcohol drinking in mice,[60] whereas Y_1 receptor activation in the paraventricular nucleus increased alcohol drinking in rats.[61] Further discussions of this topic can be found in reviews by Robinson and Thiele[62] and Thorsell and Mathé.[63]

Norepinephrine system

The locus coeruleus is densely packed with noradrenergic neurons that project to specific brain nuclei in the amygdala, prefrontal cortex, and hippocampus and that are important in the regulation of emotion and motivation.[64] Stress engages some of these projections. For example, in a study of rats, immobilization stress increased norepinephrine release in the central amygdala.[65] In a different study of the central amygdala, alpha$_1$-adrenergic receptor blockade with prazosin reduced stress-induced augmentation of anxiety-like behavior.[66] Research has also demonstrated that prazosin blocked stress-induced reinstatement of alcohol-seeking in rats.[67] In a study of rats chronically exposed to high-dose alcohol, administration of prazosin[68] or the beta-adrenergic receptor blocker propranolol[69] blocked escalation of alcohol drinking during alcohol withdrawal.

Stress and chronic alcohol exposure also increase the activity of the sympathetic nervous system

(a subdivision of the autonomic nervous system, which mediates the flight-or-fight response) and thereby affect the function of many organ systems, in part through increased noradrenergic signaling. For example, psychosocial stress in mice has been shown to increase blood pressure via an alpha$_1$-adrenergic receptor–dependent mechanism.[70]

During withdrawal from chronic, high-dose alcohol exposure, increases in sympathetic activity contribute to aversive physiological symptoms, such as increased blood pressure, heart rate, and sweating, which are thought to contribute to relapse in abstinent individuals.[71] In studies of rats, blockade of alpha$_1$- and beta-adrenergic receptors[72,73] and activation of alpha$_2$-adrenergic autoreceptors[73] reduced alcohol withdrawal symptoms such as convulsions, tremors, and locomotor hyperactivity. In another study of rats, blockade of norepinephrine signaling during withdrawal attenuated alcohol drinking.[68] See the review by Vazey and colleagues[74] for further discussion of this topic.

Conclusion and Future Directions

Brain stress systems mediate the effects of stress on alcohol drinking and the effects of chronic alcohol exposure on subsequent alcohol drinking and stress reactivity. Therefore, brain stress systems are useful targets for the development of medications for AUD and for co-occurring stress-related disorders. More specifically, glucocorticoid, CRF, dynorphin, neuropeptide Y, and norepinephrine systems may be useful targets for modulating stress-alcohol interactions. Several pharmacological agents that target these systems are promising candidates for the treatment of AUD and co-occurring mental health conditions in humans.[75] In addition, emerging evidence has shown that several other brain stress signaling systems, such as oxytocin,[76] nociceptin,[77,78] and neuropeptide S,[79] also contribute to stress-alcohol interactions, suggesting they also may be promising therapeutic targets. To guide medications development for AUD and co-occurring stress-related disorders, future studies should elucidate the mechanisms through which stress-related neuropeptide and neurotransmitter systems affect alcohol- and stress-related behaviors, including how these systems interact or modulate glutamate and gamma-aminobutyric acid (GABA) neurotransmission in specific circuits.[80,81]

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Pharmacotherapy for Co-Occurring Alcohol Use Disorder and Post-Traumatic Stress Disorder

Targeting the Opioidergic, Noradrenergic, Serotonergic, and GABAergic/Glutamatergic Systems

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Alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD) are highly comorbid, and treatment outcomes are worse in individuals with both disorders. Several neurobiological systems have been implicated in the development and maintenance of AUD and PTSD, and pharmacologic interventions targeting these systems for singular diagnoses of AUD or PTSD have proven effective. However, there are no established treatments for co-occurring AUD and PTSD, and relatively few studies have examined potential pharmacotherapy for treating symptoms of both AUD and PTSD in comorbid populations. This review provides a brief overview of the studies to date on pharmacotherapeutic treatment interventions for comorbid AUD and PTSD and highlights future directions for promising targets that have potential in the treatment of individuals with this dual diagnosis. Clinical implications of these findings are also discussed. While current medications targeting the opioidergic, noradrenergic, serotonergic, and GABAergic/glutamatergic brain systems are only modestly efficacious in improving symptoms in individuals with comorbid AUD and PTSD, novel targets within these neurobiological systems may be clinically useful for treating alcohol use outcomes and PTSD symptom severity. More work is needed to optimize pharmacologic treatment strategies that target both alcohol-motivated behavior and PTSD-related symptoms in individuals with co-occurring AUD and PTSD.

KEY WORDS: **alcohol; alcohol use disorder (AUD); comorbidity; pharmacotherapy; post-traumatic stress; post-traumatic stress disorder (PTSD)**

Introduction

Over the past decade, 12-month alcohol use, high-risk drinking, and alcohol use disorder (AUD) have increased by 11.2%, 29.9%, and 49.4%, respectively, in the United States.[1] In addition to increasingly high prevalence rates of AUD and the severe health and economic consequences associated with the disorder,[2] AUD is also highly comorbid with other psychiatric illnesses. One such comorbidity is post-traumatic stress disorder (PTSD). PTSD is a chronic and disabling disorder and is characterized by intrusive or distressing thoughts, persistent avoidance of stimuli related to the traumatic event, negative alterations in cognition or mood, and symptoms of arousal following exposure to a traumatic event. Lifetime and 12-month prevalence of PTSD in the general population are 6.1% and 4.7%, respectively.[3] This percentage is larger in certain populations, such as veteran populations, where lifetime prevalence ranges from 6.9% in U.S. veterans to 37.3% in war-specific cohorts.[4] Previous estimates suggest that individuals with PTSD are more likely to have comorbid AUD, as much as 42% of individuals within the general population[5] and 55% of veterans.[4] This is consistent with recent epidemiologic findings demonstrating a reciprocal relationship between the two disorders, such that the odds of having PTSD are significantly greater in individuals with lifetime AUD.[6]

Individuals with both AUD and PTSD typically exhibit worse outcomes, ranging from social consequences and psychological problems to treatment responses, when compared with individuals with either diagnosis alone.[7] Individuals with comorbid AUD and PTSD tend to have more severe PTSD symptoms, increased alcohol-related problems, increased risk of relapse, more frequent hospitalizations, increased emotional dysregulation, and increased odds of additional psychiatric comorbidity and suicide attempts than individuals with either disorder alone.[8,9] Other difficulties in this comorbid population include increased unemployment and homelessness. To further complicate the picture, only 19.8% and 59.4% of those with singular diagnoses of lifetime AUD and PTSD, respectively, ever seek or receive treatment,[3,6] and treatment-seeking rates in individuals with comorbid AUD and PTSD are very low.[8] Treatment adherence and response are also poorer in individuals with both disorders, compared with individuals with a singular diagnosis.[9]

The neurobiology underlying AUD and PTSD is complex and not fully understood. While not comprehensive of all systems, the opioid, norepinephrine, serotonin, gamma-aminobutyric acid (GABA), and glutamate neurotransmitter systems are independently implicated in the pathophysiology of the development and maintenance of both AUD and PTSD.[9,10] Extensive research has focused on the opioidergic system specifically for AUD[11] and to a lesser extent for PTSD.[12] More recent attention has focused on the importance of the role of brain stress systems in both drinking behavior[13] and PTSD symptomology,[14] highlighting the importance of the noradrenergic system. "Feed-forward" mechanisms within the stress systems may mediate exaggerated stress responses in individuals with AUD and PTSD. In brief, corticotropin-releasing hormone stimulates the release of norepinephrine in response to stress.[15] Increased levels of norepinephrine are thought to play an important role in arousal, drug-motivated behaviors, withdrawal, and PTSD. Further, norepinephrine release and stress can lead to the release of serotonin,[15] which is commonly associated with anxiety disorders and depression but also PTSD. Recent evidence suggests that GABAergic and glutamatergic pathways may also be linked to AUD and PTSD. GABA and glutamate work synergistically and are important in neural plasticity, memory consolidation, fear learning, anxiety, and drug craving,[16] lending support for these systems in the maintenance of AUD and PTSD. Targeting alcohol responses and stress reactivity within these systems to treat comorbid AUD and PTSD represents a niche area of research and warrants further investigation.

Although several thorough reviews on interventions for comorbid AUD and PTSD have been published recently,[16] this review aims to discuss pharmacotherapy for comorbid AUD and PTSD in terms of five neurobiological systems: the opioidergic, noradrenergic, serotonergic, GABAergic, and glutamatergic systems. While not comprehensive of all systems that may be dysregulated by both AUD and PTSD, most of the existing work examining pharmacologic treatments in individuals with comorbid AUD and PTSD have focused on these neurobiological systems. To date, there are 12

studies, including randomized controlled trials, small open-label trials, and retrospective studies, that have examined pharmacotherapy targeting opioidergic, noradrenergic, serotonergic, and GABAergic/glutamatergic systems for the treatment of co-occurring AUD and PTSD. These studies, reviewed in this article, indicate that there is limited to modest efficacy in reducing both alcohol use outcomes and symptoms associated with PTSD in individuals with a dual diagnosis. Because effective pharmacologic treatments remain elusive, finding novel treatment targets or pharmacotherapeutic treatment strategies for comorbid AUD and PTSD is critical.

The purpose of this review is to provide an overview of current clinical trials and human experimental studies examining pharmacotherapy for comorbid AUD and PTSD. For each neurobiological system discussed, we provide potential candidates that could be examined in future studies on effective treatment targets. Finally, we provide future research directions and suggestions that have potential to advance the field toward improvements in clinical treatment options for individuals with both AUD and PTSD. While there is a rich literature on behavioral treatments for comorbid AUD and PTSD, behavioral interventions are beyond the scope of the present review (see Simpson, Lehavot, and Petrakis for a review of behavioral clinical trials).[17]

Agents Acting on the Opioidergic System

Naltrexone, a nonselective opioid antagonist that is one of four U.S. Food and Drug Administration (FDA)-approved medications to treat AUD, was approved based on two randomized controlled trials that demonstrated reductions in alcohol craving, drinking days, and risk to alcohol relapse.[10] Few studies have examined naltrexone for PTSD without comorbidity, and results are mixed and limited by small sample sizes.[12] To date, three studies have examined oral naltrexone for treating co-occurring AUD and PTSD,[18-20] demonstrating modest efficacy on alcohol use outcomes and craving and limited efficacy for improving some PTSD symptoms. In veterans with comorbid AUD and PTSD, naltrexone, when compared with placebo, effectively reduced the percentage of heavy-drinking days and increased consecutive days of abstinence.[18] But in a separate study of veterans with comorbid AUD and PTSD, naltrexone given in addition to paroxetine or desipramine, serotonin and norepinephrine reuptake inhibitors, respectively, decreased alcohol craving but did not influence drinking outcomes.[19] Both studies used 50 mg/day naltrexone, and the latter study did not examine naltrexone alone.

One other study examined 100 mg/day naltrexone in both civilians and veterans with comorbid AUD and PTSD.[20] In that study, naltrexone, relative to placebo, decreased alcohol craving and the percentage of drinking days. PTSD symptom severity declined over the course of all three studies, but there was no advantage of naltrexone over placebo. Further, in an exploratory analysis, Foa and colleagues demonstrated that individuals treated with naltrexone and prolonged exposure therapy were more likely to have a clinically meaningful reduction in PTSD symptom severity at 6-month follow-up, compared with the other three treatment conditions: placebo plus prolonged exposure therapy, naltrexone plus supportive counseling, or placebo plus supportive counseling.[20] It should be noted that these studies were conducted with veterans and civilians who had a dual diagnosis of AUD and PTSD, suggesting efficacy across multiple populations.

Other Opioidergic Medications

Naltrexone was efficacious in reducing alcohol use outcomes but did not consistently or robustly improve PTSD symptoms in individuals with AUD and PTSD. Other medications targeting the opioidergic system show promise in reducing symptoms associated with singular diagnoses of AUD or PTSD, but these medications have yet to be tested in individuals with AUD and PTSD comorbidity. For alcohol, randomized controlled trials demonstrate that nalmefene, a combined mu-opioid receptor antagonist and partial kappa-opioid receptor agonist, is effective in reducing a number of alcohol use outcomes, compared with placebo, in individuals with AUD (see Mann et al. for a review).[21] Older studies have also evaluated nalmefene for PTSD, with some indication that nalmefene reduces emotional numbing, nightmares, flashbacks, intrusive thoughts, and other PTSD-associated symptoms.[22] However, to date, no studies

have examined nalmefene for comorbid AUD and PTSD.

Other findings suggest that signaling at primarily kappa-opioid receptors plays a role in alcohol-motivated behaviors. Preclinical studies suggest that the kappa-opioid receptor antagonists JDTic and nor-binaltorphimine (nor-BNI) attenuate alcohol self-administration and cue-induced reinstatement of alcohol-seeking in rodents, with some indication that kappa-opioid receptor antagonists are more effective in alcohol-dependent versus nondependent animals.[23] Kappa-opioid receptors are also thought to play a role in regulating stress and anxiety, and they have been suggested for use as pharmacologic agents for the treatment of stress-related psychiatric disorders.[24] Because kappa-opioid receptor antagonists have the ability to reduce persistent hyperarousal and improve alterations in cognition, characteristic symptoms of PTSD, they may be useful for this clinical indication. Unfortunately, not many studies have examined these pharmacologic treatments for AUD or PTSD alone or for their comorbidity. Targeting kappa-opioid receptors may be a promising avenue for individuals with AUD and PTSD, especially for individuals with severe AUD, as JDTic was more effective in alcohol-dependent rodents than in nondependent rodents.

Agents Acting on the Noradrenergic System

Prior studies evaluating the efficacy of prazosin, an alpha$_1$-adrenergic antagonist, for separate indications of AUD[25,26] and PTSD[27] have demonstrated promising results in reducing alcohol- and PTSD-related outcomes, respectively. However, to date, only two studies have evaluated prazosin for co-occurring AUD and PTSD, with mixed results. In the first study, a 6-week, placebo-controlled trial of 16 mg/day of prazosin was effective in reducing percent drinking days per week and percent heavy-drinking days per week in civilians and veterans with comorbid AUD and PTSD.[28] Results also showed a trend toward reduced alcohol craving. In the second study, the same dose of prazosin (16 mg/day) was not advantageous over placebo in reducing drinking in veterans with comorbid AUD and PTSD, although drinking did decline over the course of the 12-week study overall.[29] This study was conducted at two different Veterans Health Administration (VHA) outpatient sites, and alcohol use outcomes were confounded by a site difference, such that better outcomes were demonstrated at the VHA site providing sober housing during treatment. In both studies, prazosin was not more effective than placebo in improving PTSD symptoms or symptom severity.

One other study examined the noradrenergic antidepressant desipramine, a norepinephrine reuptake inhibitor, among veterans with comorbid AUD and PTSD.[19] In this clinical trial, which did not include a placebo-only control group, desipramine, versus the serotonergic antidepressant paroxetine, decreased the number of drinks per drinking day and the percentage of heavy-drinking days. Like the two prazosin studies, there was a decrease in PTSD symptoms over time but no significant differences between medications.

Other Noradrenergic Medications

Of the two studies that evaluated prazosin for co-occurring AUD and PTSD, only one found an effect on drinking behavior,[28] and neither found an effect on PTSD outcomes.[28,29] While this is discouraging, a recent human laboratory study indicated that doxazosin, another alpha$_1$-adrenergic antagonist, was effective in reducing alcohol consumption in individuals with AUD who had a strong family history of alcohol problems.[30] Studies on doxazosin for PTSD also indicate that the drug may be effective in reducing some PTSD symptoms.[31] Doxazosin is also currently being studied in individuals with comorbid AUD and PTSD. Doxazosin may be more advantageous than prazosin for the treatment of either indication alone, or their comorbidity, due to the long-acting nature of the drug. Doxazosin has a half-life of approximately 18 hours, whereas prazosin has a half-life of approximately 2 to 4 hours. Thus, medication adherence and study retention may improve due to a once-daily dosing schedule of doxazosin compared with multiple prazosin doses throughout the day.

Like prazosin and doxazosin, propranolol also targets the noradrenergic system, but at beta-adrenergic receptors, and it may be a treatment option for individuals with comorbid AUD and PTSD. While limited, studies in humans have shown

that propranolol reduces alcohol craving and somatic symptoms associated with alcohol withdrawal,[32] and previous literature has demonstrated the efficacy of propranolol in reducing intrusive traumatic memories and flashbacks associated with PTSD.[33]

More recently, there has been interest in the ability of propranolol to disrupt drug-related memory reconsolidation, which may be effective in reducing rates of drug relapse. In rodents, repeated propranolol administration disrupted the memory for alcohol-cue associations, such that animals reduced responding for alcohol,[34] but results have not been consistent.[35] In humans, propranolol decreased drug craving when administered before memory reactivation through a script detailing a personalized drug-taking experience.[36] However, like the preclinical findings, studies in humans have had mixed results regarding propranolol's ability to disrupt drug-associated memory reconsolidation.[37] Also, to our knowledge, propranolol has not yet been tested specifically in humans for alcohol-associated memories.

Propranolol has also been tested for its ability to disrupt trauma-related memories. Evidence suggests that propranolol effectively reduces physiologic reactivity, fear-rated memories associated with trauma, and PTSD severity, if given soon after a traumatic event,[38] and it may be used as a strategy to reduce the development or severity of PTSD.[39] Because propranolol demonstrates efficacy in reducing alcohol-motivated behavior, attenuating PTSD symptoms, and disrupting both drug- and trauma-associated memory reconsolidation, propranolol may also be effective in reducing alcohol use outcomes and PTSD symptom severity in individuals with comorbid AUD and PTSD, providing another potential avenue for treatment and clinical improvement.

Agents Acting on the Serotonergic System

Selective serotonin reuptake inhibitors (SSRIs) have been the first-line of treatment for PTSD, with only two SSRIs FDA-approved to treat PTSD—sertraline and paroxetine.[40] However, the efficacy of SSRIs in treating PTSD and associated symptoms is limited, with less than 20% to 30% of patients achieving full remission.[41] Similarly, findings on SSRIs for the treatment of AUD or associated symptoms are limited.[42] To date, few studies have examined the effect of SSRIs on comorbid PTSD and AUD conditions. In the 1990s, Brady and colleagues conducted a small open-label pilot study of 200 mg/day of sertraline in individuals with comorbid PTSD and AUD.[43] Participants self-reported alcohol consumption, and the researchers found that sertraline effectively reduced PTSD symptoms and the average number of drinks consumed, and it increased the number of days of alcohol abstinence. Following these positive preliminary findings, larger trials generally have been less successful at using sertraline to treat alcohol-motivated behavior and have had only modest success using sertraline to treat PTSD.[44,45] In these trials, individuals with comorbid AUD and PTSD demonstrated decreases in drinking behavior, but sertraline was no more effective than placebo at influencing alcohol use outcomes.

Regarding PTSD, Brady and colleagues demonstrated a trend such that sertraline decreased PTSD symptom severity and the cluster symptoms of hyperarousal and intrusion to a greater degree than placebo.[44] Supporting these findings, Hien and colleagues demonstrated greater reductions in PTSD symptoms at the end of treatment for the sertraline-treated group compared with the placebo group,[45] and this effect was sustained at 6- and 12-month follow-up. Interestingly, when treated with sertraline, a subgroup of individuals with early-onset PTSD and less severe AUD had more improvement in alcohol use outcomes than individuals treated with sertraline who had late-onset PTSD and more severe AUD.[44] Further, a subsequent secondary data analysis concluded that improved PTSD symptoms, particularly hyperarousal, were associated with improved alcohol-related symptoms,[46] possibly suggesting that treatments targeted at reducing hyperarousal or hyperreactivity may be more beneficial in reducing symptoms of both AUD and PTSD in this comorbid population.

Another study examined an FDA-approved medication for the treatment of PTSD in veterans with a dual diagnosis of AUD and PTSD.[19] Paroxetine was not better than desipramine in reducing percent heavy-drinking days or drinks per drinking day, but paroxetine was comparable to desipramine in reducing PTSD symptoms. As previously discussed, naltrexone in addition

to paroxetine or desipramine reduced alcohol craving, but there was no significant additive effect of naltrexone in combination with paroxetine or desipramine on drinking or PTSD symptoms.

Finally, although not an open-label or randomized controlled trial, a retrospective study evaluated the efficacy of quetiapine, an atypical antipsychotic with antagonist effects at serotonin 5-HT_2 receptors, among veterans with AUD, of whom 90% were diagnosed with PTSD.[47] These veterans had been treated with quetiapine for sleep disturbances, as older and more recent work has shown that quetiapine is effective in reducing disturbed sleep and other symptoms associated with PTSD.[48,49] This retrospective study aimed to target alcohol use outcomes, thus changes in PTSD symptom severity were not reported. Quetiapine, when compared with placebo, decreased the number of times admitted for detoxification, increased the total number of days abstinent from alcohol use, and trended toward increasing time to relapse. While quetiapine reduced alcohol craving and alcohol consumption in individuals with AUD in preliminary human laboratory, open-label, and retrospective studies, it was not efficacious in reducing drinking outcomes in a large, multisite clinical trial.[50]

Other Serotonergic Medications

As previously mentioned, sertraline and paroxetine are the only two FDA-approved medications to treat PTSD, and evidence suggests that these medications target PTSD symptom severity, versus the overall reduction or remission of PTSD symptoms, in individuals without AUD and PTSD comorbidity.[51] Further, based on findings in this review, sertraline yields mixed results in comorbid populations regarding the reduction of alcohol use outcomes and PTSD symptoms. Trazodone, a second-generation antidepressant and antagonist at serotonin 5-HT_2 and alpha$_1$-adrenergic receptors, is prescribed off-label for singular AUD or PTSD and may be an effective second-line treatment for individuals with co-occurring AUD and PTSD. Likely due to trazodone's anxiolytic- and sedative-like properties, early studies demonstrated that trazodone improved sleep disturbances associated with AUD and alcohol withdrawal.[52] However, in a study of alcohol detoxification patients, the trazodone-treated group increased alcohol consumption following cessation of the medication.[53]

Regarding PTSD, older studies demonstrated that trazodone decreased PTSD symptoms and dysregulated sleep associated with PTSD.[54] In individuals with co-occurring substance abuse and anxiety symptoms, including PTSD symptoms, trazodone decreased alcohol consumption and reduced anxiety symptoms.[55] While trazodone has not yet been investigated in individuals with comorbid AUD and PTSD, and recently published studies on the efficacy of trazodone for either indication remain elusive, there is some evidence suggesting that trazodone may be clinically useful for treating sleep disturbances associated with both AUD and PTSD and possibly their comorbidity. However, results should be interpreted with caution until further studies can establish the safety and efficacy of trazodone in AUD and PTSD populations.

Further, 3,4-methylenedioxy-methamphetamine (MDMA) has shown promise for treatment-resistant and chronic PTSD.[56,57] MDMA, a derivative of methamphetamine, primarily acts to increase the net release of serotonin, although it may stimulate the release of other monoamine neurotransmitters (dopamine and noradrenaline) as well. Pilot studies and a long-term follow-up study demonstrated that MDMA-assisted psychotherapy reduced PTSD symptoms and increased self-reported improvement in individuals with resistant, chronic PTSD.[58] While these results are encouraging for PTSD, to our knowledge, MDMA has not been investigated as a treatment for AUD or comorbid AUD and PTSD. The abuse liability of MDMA may make it less desirable as a medication for the treatment of any substance use disorder (SUD), including AUD.

Agents Acting on the GABAergic and Glutamatergic Systems

There is promising evidence suggesting that the GABA and glutamate systems may be targets for treating comorbid AUD and PTSD.[59] While not FDA-approved for the treatment of AUD, topiramate, an anticonvulsant with action at both GABA and glutamate receptors, has demonstrated efficacy in reducing alcohol consumption in humans and is recommended as a second-line treatment.[10]

Furthermore, other studies suggest that topiramate may be effective in treating PTSD.[60] Contributing to the framework for studying topiramate in this comorbid population, an 8-week, open-label pilot study assessed the effect of topiramate among veterans with PTSD.[61] These veterans did not have co-occurring AUD and PTSD, but the authors examined the effect of topiramate on alcohol use and PTSD symptoms. In this study, topiramate was effective in reducing drinking behavior in individuals with high-risk drinking patterns, as well as reducing nightmares and sleep disturbances associated with PTSD. Because the results from this pilot trial and other research demonstrated the efficacy of topiramate for either AUD or PTSD, Batki and colleagues conducted the first randomized controlled trial of topiramate among veterans who have comorbid AUD and PTSD.[62] Topiramate, when compared with placebo, was effective in decreasing alcohol craving and the percentage of drinking days, and topiramate trended toward decreasing PTSD symptom severity and hyperarousal. It should be noted that there were significant cognitive effects of topiramate on learning and memory in this study, but these cognitive deficits improved by the end of treatment.

Other GABAergic and Glutamatergic Medications

Zonisamide is an anticonvulsant agent similar to topiramate, but it may have fewer side effects. This may be due to the more indirect effect of zonisamide on GABA and glutamate activity, compared with topiramate.[63] A small study evaluating the efficacy of zonisamide in the treatment of AUD showed that zonisamide was well-tolerated and reduced heavy-drinking days, drinks per week, and alcohol urges,[63] and a small pilot study suggests its safety in comorbidity (I. L. Petrakis, personal communication, 2018).

Gabapentin and pregabalin, other FDA-approved anticonvulsants exerting action on GABA synthesis in the brain, have been studied to a moderate extent for their potential in treating AUD and alcohol withdrawal syndrome.[64] In individuals with AUD, gabapentin effectively reduced heavy drinking and alcohol craving, and it improved rates of abstinence,[65] although results are mixed, with some findings indicating that gabapentin is more efficacious in individuals with a history of alcohol withdrawal.[66] Pregabalin is more potent than gabapentin and also has positive effects on alcohol craving and withdrawal.[67] Because of the anxiolytic properties of both drugs, including their role in reducing generalized anxiety, these agents may hold promise in diminishing symptoms associated with PTSD. Some case reports and retrospective studies confer an advantage of gabapentin over placebo in reducing flashbacks, nightmares, and other sleep disturbances.[68,69] In a randomized controlled trial and case report, pregabalin, when administered in addition to standard medication, also improved PTSD symptom severity, hyperarousal, and sleep disturbances in individuals with combat-related PTSD or sexual trauma.[70,71] While these anticonvulsants have modest efficacy in reducing drinking behavior and PTSD symptoms independently, they should not be ruled out as secondary treatment options for individuals with co-occurring AUD and PTSD who are unresponsive to first-line treatments, especially for individuals who have alcohol withdrawal syndrome or sleep problems associated with PTSD.

Recent evidence also suggests a role for the metabotropic glutamate receptor 5 (mGluR5) in the pathophysiology of PTSD and AUD. Preclinical studies indicate that mGluR5 activity may mediate fear conditioning[72] and regulate alcohol-related behavior.[73] Indeed, antagonists at mGluR5 sites, such as 2-methyl-6-(phenylethynyl)-pyridine (MPEP), block the acquisition of fear and decrease alcohol self-administration and reinstatement in rodents.[73,74] In humans, new positron emission tomography (PET) neuroimaging results demonstrate higher mGluR5 availability and positive correlations between mGluR5 availability and avoidance symptoms in individuals with PTSD.[75] This makes sense, considering that the preclinical literature indicates that mGluR5 receptors are involved in the regulation of fear and stress-related behaviors.[72] Likewise, hyperactivity at glutamatergic receptors is associated with chronic alcohol misuse,[76] and PET studies have demonstrated alterations in mGluR5 availability in individuals with AUD, including those who are abstinent.[77]

Taken together, blocking mGluR5 sites may be beneficial in reducing both PTSD-related symptoms

and alcohol use outcomes in individuals with both disorders. Although not yet empirically tested, mGluR5 antagonism could provide another new approach for treating comorbid AUD and PTSD. It should be noted that there may be unwanted effects associated with GABAergic or glutamatergic medications, namely cognitive impairment.[62,76] Therefore, treatment approaches involving drugs targeted at the GABA or glutamate neurotransmitter systems may be warranted only in individuals unresponsive to other treatment options.

Other Targets

Neurokinin-1 receptors have also been targeted as having an effect on alcohol-motivated behavior because of their role in the stress response, with results indicating efficacy in reducing alcohol craving and cortisol reactivity in humans[78] and in blocking stress-induced reinstatement of alcohol-seeking in rodents.[79] However, in a human experimental study of individuals with co-occurring AUD and PTSD, aprepitant, a neurokinin-1 receptor antagonist, demonstrated no advantage over placebo in decreasing alcohol craving, subjective responses to stress or alcohol cues, or PTSD symptom severity.[80]

Other treatment targets may include the antioxidant *N*-acetylcysteine, the novel vasopressin 1b receptor antagonist ABT-436, and the neuropeptide oxytocin. A recent pilot trial examined the effect of *N*-acetylcysteine or placebo in veterans with comorbid PTSD and SUD and found *N*-acetylcysteine to be more effective than the placebo in reducing drug craving and PTSD symptoms.[81] Preclinical work has shown that *N*-acetylcysteine reduced alcohol-seeking and reacquisition of alcohol self-administration in rodents.[82] Another recent clinical trial examined the effect of ABT-436 in individuals with AUD only and found that ABT-436, when compared with placebo, increased days of abstinence.[83] Importantly, in a subgroup analysis, individuals with higher baseline levels of stress demonstrated better ABT-436 treatment responses for drinking outcomes. Thus, individuals with AUD and high stress may benefit most from vasopressin 1b antagonism, likely indicating that ABT-436 may be an effective, promising pharmacologic treatment option for individuals with comorbid AUD and PTSD.

Because of its anxiolytic properties,[84] oxytocin also presents as a potential candidate for the treatment of PTSD[85] and AUD.[86] In patients with PTSD, oxytocin decreased total PTSD symptoms provoked by exposure to a traumatic script, the intensity of recurrent thoughts about trauma, subjective anxiety and tension, and amygdala reactivity to emotional faces.[87] Oxytocin also reduced alcohol withdrawal in patients with AUD,[88] and it may moderate cue-induced alcohol craving in a subset of individuals who have anxiety and AUD.[89] To our knowledge, oxytocin has yet to be tested in a comorbid population. These avenues should be explored in future investigations.

Combination Pharmacotherapies

Combination pharmacotherapy may be another viable treatment option for co-occurring AUD and PTSD, as the clinical efficacy of monotherapy is limited to modest in treating both alcohol use and PTSD symptoms in this comorbid population. In preclinical studies, prazosin, naltrexone, and propranolol all singularly reduced responding for alcohol and decreased alcohol self-administration, but these drugs also reduced other palatable, oral reinforcers.[90] Subthreshold dosing combinations can be used on the basis that a combination of already efficacious medications can target multiple neural systems. Or, combined medications can target one neural system but affect different receptor subtypes that may be dysregulated in each disorder, thus addressing different symptoms or aspects of behavior. Similarly, medications with different mechanisms of action can be used in combination and in a lower dose range to potentially minimize side effects associated with higher doses of one drug alone, possibly improving medication compliance and study retention.[91]

Work in rodents confirms that combination pharmacotherapy may be a promising treatment approach for AUD. When administered in combination, prazosin and propranolol, two drugs targeting different receptor subtypes within the same neural system, were more effective than either drug alone in decreasing alcohol intake.[90,92] Further, prazosin in combination with naltrexone, two drugs targeting different neural systems, was more effective

in reducing alcohol-seeking and consumption than either drug alone.[90,93]

This combination approach has also been proposed as a treatment strategy for PTSD to optimize treatment response and prevention.[33] Medications within the noradrenergic system but with differing mechanisms of action have been shown to treat separate symptoms of PTSD. For example, prazosin, the alpha$_1$-adrenergic receptor antagonist, reduces combat-related nightmares and insomnia; whereas propranolol, the beta-adrenergic receptor antagonist, decreases flashbacks and traumatic memories associated with PTSD. As such, Shad and colleagues postulated that prazosin in combination with propranolol may lead to significant clinical improvement of PTSD by treating a broader spectrum of PTSD-related symptoms, an effect not demonstrated with monotherapy.[33]

Further, a fairly recent case report suggests that prazosin in combination with naltrexone was effective in reducing alcohol craving and PTSD-related flashbacks within 4 days of treatment, with complete remission of alcohol craving and flashbacks in 2 to 4 weeks.[94] It should be noted that these findings were from a single male subject diagnosed with AUD, PTSD, and bipolar II disorder who was taking lithium concurrently with prazosin and naltrexone. To our knowledge, combination pharmacotherapy targeting the noradrenergic system has not yet been tested in human laboratory studies or pilot trials of individuals with co-occurring AUD and PTSD and may be one possible direction to guide optimal and novel clinical treatment approaches for this vulnerable comorbid population.

Clinical and Research Implications

To date, only 12 studies have examined pharmacologic treatment for co-occurring AUD and PTSD. Three studies targeted mainly the opioidergic system, two targeted the noradrenergic system, four targeted the serotonergic system, two targeted the GABAergic and glutamatergic system, and one targeted the neurokinin-1 receptor. Consistent with conclusions from the recent comprehensive review by Petrakis and Simpson,[16] there are contradictory findings within each neurobiological system targeted. Overall, findings within the opioidergic system demonstrated a modest reduction in alcohol use outcomes. Prazosin, a target within the noradrenergic system, yielded mixed results regarding alcohol use, and neither of the two studies found an effect on PTSD outcomes. Serotonergic medications also yielded mixed results on alcohol use outcomes but tended to improve PTSD symptoms overall. Topiramate, acting at both GABA and glutamate receptors, reduced drinking behavior and improved PTSD symptoms. While topiramate may stand out as the most promising medication for comorbid AUD and PTSD, larger studies need to be conducted to evaluate its safety and efficacy, especially given the cognitive side effects of the drug. Future work should consider investigating lower doses of topiramate to decrease side effects and improve personalized medicine.[95]

Several factors may contribute to the overall mixed results. Sample sizes were relatively small for half of the studies. While some studies included women, others examined only men or few women. This gender gap could be problematic, as recent research indicates that medication response may differ by gender for naltrexone, some serotonergic medications, and noradrenergic targets. For example, in one study, women's responsiveness to naltrexone varied across the menstrual cycle, and, during the luteal and early follicular phases, treatment with naltrexone increased serum cortisol,[96] which may have implications for stress reactivity in both AUD and PTSD. Other research suggests that women have better treatment responses to SSRIs, including sertraline, and have fewer associated adverse events.[97]

Recent evidence also suggests that noradrenergic targets for tobacco dependence may differentially attenuate stress reactivity in women and nicotine-related reinforcement in men.[98] It is plausible that noradrenergic compounds may also preferentially target gender-sensitive systems for AUD and may be more effective in treating women with post-traumatic stress. Further, recent findings suggest that the prevalence of drinking has increased among women over the past decade,[1] and women have higher rates of PTSD than men.[3] Thus, it is important to consider sample size and the ability to detect gender differences in medication response when examining pharmacotherapies for comorbid AUD and PTSD, especially given that many studies were conducted primarily in males.

Another challenge in treating comorbid AUD and PTSD may be related to the type of trauma endured

prior to the onset of PTSD. For example, half of the studies examining pharmacotherapy for co-occurring AUD and PTSD reviewed in this article investigated treatment effects in veterans, and many of them had combat-related trauma. The other half examined treatment effects in civilian populations with traumas resulting from childhood experiences, sexual assault, physical assault, witnessing death, and natural disasters. To further complicate treatment, at least one study demonstrated that the severity and order of the development of comorbidity may be related to treatment efficacy. Sertraline was more effective in reducing drinking outcomes in individuals with early-onset PTSD and less severe AUD than in those with late-onset PTSD or more severe AUD.[44] Thus, further research on personalizing treatment to reflect diagnostic onset and trauma type may be a relevant approach when examining novel targets or strategies for co-occurring AUD and PTSD.

Given the high rates of comorbidity for these two psychiatric disorders, it is somewhat surprising that so few studies have examined effective pharmacologic treatment options. This could be due to the complexity associated with psychiatric comorbidity and the difficulties of conducting research among this population. Treatment studies tend to focus on the effect of medication on one disorder, often excluding for comorbidity. However, real-world clinical populations often include comorbid conditions, further emphasizing the urgent need to examine better pharmacotherapies for improving co-occurring AUD and PTSD in a clinically meaningful way.

Promising targets within each system have demonstrated efficacy in treating independent diagnoses of both AUD and PTSD. For example, nalmefene, doxazosin, propranolol, trazodone, gabapentin, and pregabalin have all been found to reduce alcohol- and PTSD-related outcomes, but they have not yet been tested in comorbid populations. Further, subthreshold combination pharmacotherapy in animal models has been efficacious in reducing alcohol-motivated behavior, and may be an effective strategy for individuals who are unresponsive to first-line treatments or for those who are sensitive to adverse events associated with higher doses of a singular drug.

There is a rich literature on behavioral treatments for comorbid AUD and PTSD that is beyond the scope of the current review.[17] However, future research should also consider examining behavioral interventions in combination with these novel pharmacotherapies to better manage alcohol use outcomes and PTSD symptoms in this comorbid population. Human laboratory studies provide an efficient, cost-effective avenue for evaluating the effects of potential medications on psychiatric disorders. This method has been used effectively to screen medications for drug use disorders.[99] When examining treatments for co-occurring AUD and PTSD, investigators are encouraged to use promising treatment targets or their combinations. Also, researchers can use human laboratory paradigms to screen these potential medications in an effort to optimize the clinical utility of pharmacotherapeutic treatments for comorbid AUD and PTSD.

Conclusion

Pharmacotherapeutic treatment options for co-occurring AUD and PTSD are limited. To date, only 12 studies have examined pharmacologic interventions in this comorbid population, and most demonstrated only modest efficacy, but results are mixed. While not comprehensive of all neurobiological systems that may be dysregulated by alcohol use and post-traumatic stress, the existing literature has focused on the opioidergic, noradrenergic, serotonergic, and GABAergic/glutamatergic systems. Targeting other promising, efficacious medications within these neurobiological systems, or combining medications within the same system or across systems, may be an important and promising next step in treating comorbid AUD and PTSD, especially among individuals who are unresponsive to first-line treatments. Future studies need to urgently address this critical literature gap in order to advance pharmacotherapeutic treatment options in special populations with co-occurring AUD and PTSD.

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Alcohol Use Disorder and Traumatic Brain Injury

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Alcohol use and traumatic brain injury (TBI) are inextricably and bidirectionally linked. Alcohol intoxication is one of the strongest predictors of TBI, and a substantial proportion of TBIs occur in intoxicated individuals. An inverse relationship is also emerging, such that TBI can serve as a risk factor for, or modulate the course of, alcohol use disorder (AUD). Critically, alcohol use after TBI is a key predictor of rehabilitation outcomes, prognosis, and additional head injuries. This review provides a general overview of the bidirectional relationship between TBI and AUD and a discussion of potential neuropsychological and neurobiological mechanisms that might underlie the relationship.

KEY WORDS: alcohol and other drug use (AODU) development; AODU initiation; brain; injury; trauma

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Overview of Traumatic Brain Injury

Traumatic brain injury (TBI) is characterized by neurological dysfunction caused by a bump, blow, or penetrating injury to the brain. The duration and severity of dysfunction may range from "mild" TBI (concussion), which may involve a brief period of loss of consciousness and a transient neurological impairment with rapid recovery, to "severe" TBI, involving an extended period of loss of consciousness and permanent brain damage.[1] The extent of neurological damage is determined by an evolving pathophysiology over the hours and days following the injury, during which time brain swelling, increased intracranial pressure, and reduced cerebral blood flow contribute to the development of cognitive and functional deficits.[2] Further, the injuries can be divided into those that cause focal or penetrating damage to local brain regions versus those that result in more diffuse damage.[3] Consequently, TBI is a highly heterogeneous injury state resulting in a patient population with markedly different injuries, comorbidities, and predicted outcomes.

Public understanding of TBI is currently undergoing a shift due, in part, to recent events that have focused public and media attention on the issue.[4,5] Although these recent events, which include the emerging understanding of the role of TBI in later neurodegeneration and the

recognition of the high incidence of TBI among amateur and public athletes, as well as military personnel, represent tragedies with real human cost, they have also helped focus public attention on an ongoing public health crisis.

Annually, about 2.8 million civilians in the United States receive medical treatment for TBI, but the true incidence of TBI is actually far higher, as many TBI patients are never seen by health care providers[6,7] (although rates of emergency department visits are rising, likely due to increasing public awareness of the seriousness of TBI).[8] Even among those patients seen by medical personnel, the lack of definitive diagnostic tools, or even consensus on a definition, means that a substantial proportion of TBIs go undiagnosed.[9] Additionally, TBI was declared the signature injury among military personnel involved in the protracted conflicts in Iraq and Afghanistan (Operations Enduring Freedom, Iraqi Freedom, and New Dawn).[10] During the first 12 years of these conflicts, nearly 250,000 service members were diagnosed with TBI,[11] although the difficulties associated with reporting, identifying, and diagnosing head injuries indicate that this number likely is underestimated.

What is becoming clear, is that even relatively mild TBI can have far-reaching consequences that last well beyond the initial symptoms.[12] The long-term sequelae of TBI can include psychiatric and neurological dysfunction, as well as a whole host of nonneurological diseases. Additionally, survivors of TBI can suffer from cognitive issues and are more likely to be unemployed, socially isolated, and incarcerated.[13,14] Thus, the total cost, comprising health care dollars, loss of productivity, and quality of life, associated with TBI in the United States is substantial, with estimates of lifetime cost (in 2009 dollars) ranging from more than $75 billion to more than $200 billion.[15]

Alcohol Use Disorder Before TBI

TBI has long been closely associated with acute alcohol intoxication. Most studies estimate that between 30% and 50% of patients treated for TBI were intoxicated at the time of injury, with even greater intoxication estimates for patients injured in motor vehicle accidents and assaults.[16] Binge drinking is a major risk factor for trauma, particularly brain trauma.[17] Individuals who consume more than five drinks in a sitting are more than three times as likely to suffer a trauma.[18] One illustrative example involves cyclists. Individuals who cycle while intoxicated are more likely to fall, and, among cyclists who fall, being intoxicated greatly increases the probability of TBI.[19] The lifetime incidence of TBI is approximately four times higher among individuals who drink, relative to those who do not.[20]

Not surprisingly, given the powerful relationship between alcohol intoxication and brain injuries, the overall rate of alcohol use disorder (AUD) is very high among patients who incur TBI, with estimates ranging from one-third to half of all patients meeting diagnostic criteria for AUD.[21] More than half the patients admitted for rehabilitation following TBI meet the diagnostic criteria for AUD[22] or are considered at risk for problem drinking because of self-reported binge drinking or Short Michigan Alcoholism Screening Test (SMAST) scores.[21] Thus, the population of persons with TBI disproportionately consists of individuals who drink alcohol and those who meet AUD diagnostic criteria or are at risk for developing AUD.

Given that alcohol intoxication is a major risk factor for the incidence of TBI, a substantial population exists from which researchers can study the effects of blood alcohol concentration at time of injury on survival and on functional outcomes. There is controversial literature (beyond the scope of the current review) suggesting that better long-term outcomes are associated with patients who had low to moderate levels of alcohol in their blood at the time of their injuries, when compared with patients who had no alcohol in their blood,[23,24] although not all studies have reached that conclusion.[25] What is much clearer, however, is that drinking *after* TBI represents a major impediment to successful outcomes in several critical domains.[16,26]

Patterns of Drinking After TBI

Alcohol use falls off immediately after TBI, and this reduction appears to be due to three factors.[21] First, many patients are advised to abstain from alcohol in the early postinjury period to reduce the likelihood of post-traumatic seizures.[27] Second, many patients with TBI have limited access to alcohol because

they are hospitalized, living with family, or admitted to an inpatient rehabilitation facility, or because they have impairments in cognition or mobility.[21] Finally, many patients, especially those whose injuries occurred secondary to intoxication, choose to use this early period to stop drinking. Indeed, involvement in car crashes increases the likelihood that patients will enter AUD treatment.[28] Some patients stop drinking permanently, but a large subset (25%, by some estimates) resumes drinking after injury, and consumption levels can rise to (or above) preinjury levels by 1 to 2 years after injury.[29] The strongest predictor of postinjury AUD is drinking before injury. Patients who scored high on the SMAST before TBI were more than 10 times likely to exhibit problem drinking after injury.[22]

There exists some controversy in the literature as to whether TBI can act as an independent risk factor for the development of AUD in adult patients who did not previously meet the diagnostic criteria for AUD.[30,31] Epidemiological studies have generally concluded that TBI does not induce new cases of AUD, but some patients return to drinking after TBI (approximately 25%, by some estimates),[21,30] and this has significant negative consequences (see **Consequences of Drinking After TBI** in this article). Still, there is reason to suspect that TBI can increase the likelihood of AUD. For instance, in one study, approximately 20% of patients who were abstainers or "light" drinkers before injury exhibited high-volume drinking after injury.[32] Similarly, among military personnel, several studies have reported that service men and women who experienced combat-related TBI were more likely than uninjured individuals to binge drink.[33] Additionally, among patients with a primary diagnosis of substance use disorder (defined as misuse of alcohol or drugs), a lifetime history of TBI is remarkably common. In one study of individuals seeking treatment for substance abuse in New York, more than 50% had a history of TBI, and nearly half had experienced more than one TBI.[34]

Still, any potential causal relationship between adult TBI and AUD has been difficult to establish for several reasons (although causality may exist). First, the TBI population disproportionately consists of people who exhibit AUD, potentially masking any relationship. Second, patients who have AUD after TBI appear more likely to be lost to follow-up in epidemiological and outcome studies.[35] Third, patients who have the most severe injuries, the subset of people with TBI who, theoretically, are most likely to develop AUD, are also the group most likely to have no access to alcohol because of disability or institutionalization.[36] Fourth, it is becoming increasingly clear that a large subset of patients treated for TBI also had previous TBI, and, as described in this article, injury during early development is a powerful risk factor for AUD.[37] Fifth, the populations most at risk for TBI, including adolescent and young adult males, risk-takers, and enlisted military personnel, are also at elevated risk for AUD.[38]

The relationship between TBI and AUD is much clearer in individuals who were injured as children. Incurring TBI during childhood increases the likelihood of later development of AUD. This relationship is easier to discern because the effects of injury on the developing nervous system can be profound,[39] and because this population is less affected by many of the confounders already discussed. Younger patients, presumably, are less likely to be experienced with alcohol or meet the diagnostic criteria for AUD.

For instance, results from the Christchurch birth cohort studies indicated that children who experienced mild TBI with hospitalization before age 5 were 3.6 times more likely to meet the *Diagnostic and Statistical Manual of Mental Disorders (Third Edition–Revised)* criteria for alcohol dependence during adolescence, when compared with those who had no similar injury.[40] A 10-year, nationwide, longitudinal cohort study in Taiwan indicated that there was a more than sixfold increase in the rate of alcohol abuse (as defined by the *International Classification of Diseases, Ninth Revision: Clinical Modification*) among patients with a history of TBI, when compared with uninjured control patients.[41] Among Canadian high school students, the odds ratio for binge drinking in the previous year (at the time of the study) was between two- and fourfold higher in students who had a history of TBI (defined as loss of consciousness or an overnight hospitalization), when compared with uninjured students.[42] Moreover, in a study of patients admitted for inpatient rehabilitation following TBI, approximately 20% of the population had experienced previous TBI, many sustained before age 16.[37] Among the patients in this study, those with a history of childhood brain injury had twice the rate

of problem alcohol use as those without previous TBI. (Problem alcohol use was defined as more than 14 drinks per week for males and 7 for females, or any incidence of binge drinking that included 5 or more drinks in a night.)

Also, TBI appears to act indirectly by limiting protective factors and increasing risk factors for incurring a subsequent TBI.[43] For instance, individuals with a history of TBI early in life are less likely to participate in extracurricular activities, finish school, marry, and be employed, and they are more likely to engage in risky behavior and experience long-term alienation from family and peer groups, all of which serve as risk modifiers for alcohol misuse.[37,44,45] TBI, particularly when it occurs in young patients, can modify the risks for development of AUD, and, among individuals who have AUD, there is a high incidence of prior TBI.

Comorbidity Among TBI, PTSD, and AUD

TBI is closely linked to post-traumatic stress disorder (PTSD), but not only because both conditions have trauma as a precipitating factor (see Figure 1). Among combat veterans who had physical trauma excluding the brain, 16% developed PTSD symptoms, whereas 44% of combat veterans with a history of TBI developed symptoms of PTSD.[46] Similar patterns have been observed among civilians.[47] Remarkably, this relationship exists even among individuals who experienced post-traumatic amnesia that prevented them from remembering the trauma.[48] The potential physiological links between the two conditions remain under investigation, but they may involve dysregulation of the hypothalamic

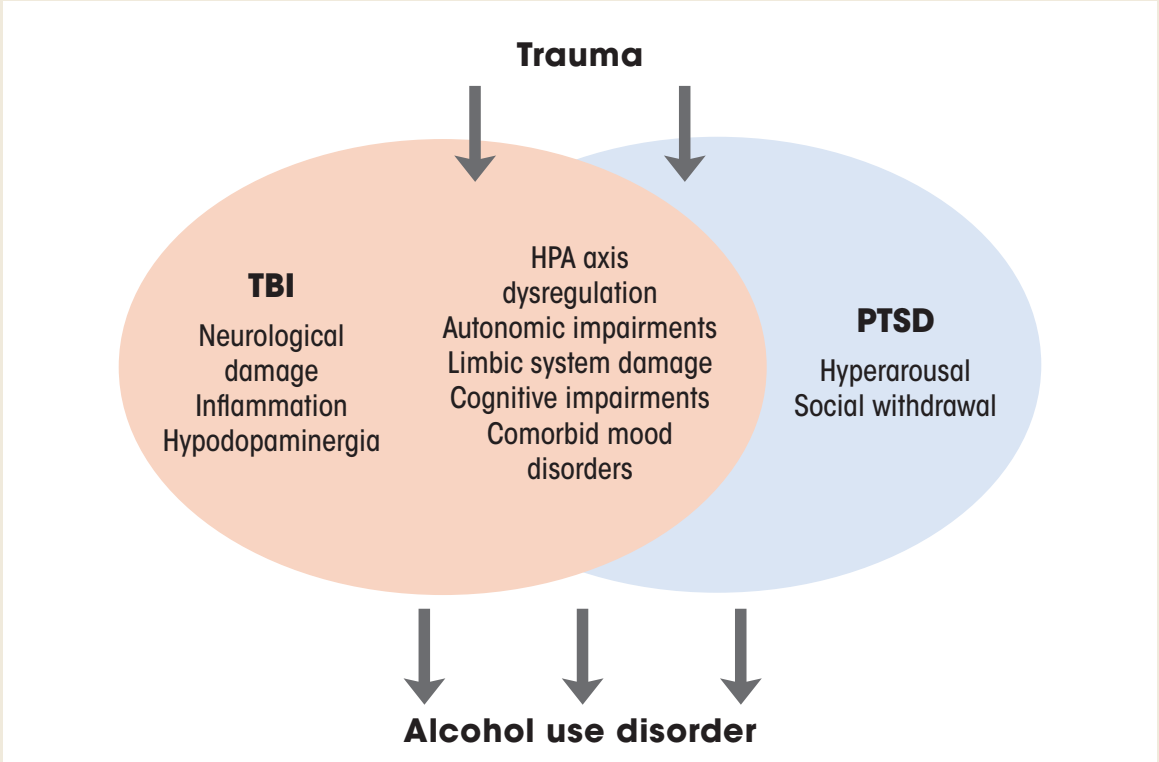

Figure 1 Overlapping neurobehavioral links among TBI, PTSD, and alcohol use disorder. TBI and PTSD share trauma as a precipitating event. They are also linked by dysregulation of stress response systems, cognitive impairments, and affective symptoms, which, together, can increase the likelihood of alcohol misuse. *Note:* HPA, hypothalamic pituitary adrenal; PTSD, post-traumatic stress disorder; TBI, traumatic brain injury.

pituitary adrenal axis, impairments in autonomic physiology, and damage to frontal and limbic structures that impair physiological regulation and the ability to manage traumatic memories.[49,50]

Critically, TBI, PTSD, and AUD are commonly comorbid, which is unsurprising given that intoxication elevates risk of TBI, and that generally high rates of alcohol misuse occur among patients who have TBI.[21] The relationships among these conditions are an area of active investigation. Numerous studies have investigated relationships between two of the conditions, and far fewer have investigated all three.[51] There are clearly relationships between and among all these conditions, but there are a number of overlapping characteristics of individuals with PTSD and TBI that can make drinking more likely.[52] For instance, the hyperarousal to stressful events that is central to PTSD pathology is unpleasant and can increase social withdrawal, thus exacerbating ongoing negative affect.[52] TBI can make it more difficult for patients to manage these symptoms, increasing the likelihood that they will drink alcohol. Moreover, the cognitive impairments combined with decreased frustration tolerance that are central to both TBI and PTSD can increase the likelihood that daily difficulties will lead to drinking. Because some of the relationship between TBI and AUD is likely mediated by PTSD, it has been difficult to disentangle the contribution of TBI and PTSD to the development of AUD, given their similar etiology and symptomatology. Further work is required to uncover the physiological substrates that link these conditions.

Consequences of Drinking After TBI

Multiple epidemiological studies have reported that a subset of people with TBI eventually drinks at or above preinjury levels.[20,22,31,32] This propensity to resume consuming alcohol at preinjury levels is of critical importance, because alcohol use after injury is deleterious in a number of different domains and is predictive of negative outcomes over the long term.[16]

A distinction has to be drawn between AUD and alcohol use in the absence of problem drinking. People who have brain injuries likely suffer negative consequences from patterns of drinking that would not produce significant harm in uninjured individuals. For instance, drinking can promote development of post-traumatic seizures directly and by interfering with the efficacy of prescribed antiseizure medications.[53] Critically, alcohol affects peripheral tissues, including in the liver and kidneys, and impairs wound healing, which can have outsized effects on patients recovering from trauma. Also, cognitive consequences of drinking appear to be magnified by prior TBI. For instance, patients with TBI who drank at "heavy social" levels (with a mean Alcohol Use Disorders Identification Test score of 16.9) exhibited impaired event-related potentials and greater cognitive deficits, when compared with patients who abstained.[54]

Finally, both drinking and a history of TBI are powerful risk factors for suffering subsequent head injuries.[55] Moreover, suffering TBI while intoxicated more than triples the likelihood of suffering a future TBI.[56] Repeated TBIs produce more severe long-term damage and permanent disability than a single injury.[55] Patients with TBI often report reduced tolerance to alcohol,[57] and they can also have balance problems associated with their injuries, meaning that intoxication, even at relatively low blood ethanol concentrations, can increase the risk of injury.

Patients with AUD who continue (or restart) drinking after TBI have significantly poorer long-term outcomes than patients who do not.[58] A chronic high level of drinking can be proinflammatory and deleterious to brain health and thus has the potential to impair functional recovery and further damage vulnerable and already impaired neural structures.[59] Many of the brain regions commonly injured in TBI, including the frontal and medial temporal regions, are also key sites of inflammatory reactions in people who have been drinking alcohol for a long time. Patients with TBI who were previously diagnosed with AUD and relapsed had smaller frontal gray matter volumes within the first year after injury than patients who did not relapse.[60] Finally, in a retrospective study of patients who had TBI, individuals who met the criteria for substance use disorder (including alcohol) at the time of their injuries were four times more likely to die from suicide than patients who did not meet the criteria.[61]

Some of the negative consequences of drinking after TBI may be related to treatment compliance. Patients with AUD are less compliant with TBI rehabilitation and have poorer rehabilitation outcomes than patients who do not have AUD.[16]

Patients with AUD are also more likely to have lower levels of life satisfaction.[62] Alcohol misuse also impairs reintegration into the workforce after injury. Among people who have TBI, alcohol misuse is the most commonly cited reason for termination from a vocational placement program.[63] Also, patients with TBI and AUD are more likely than patients with TBI who do not have AUD to meet the diagnostic criteria for mood disorders and less likely to return to work.[60]

Because of the many deleterious consequences associated with drinking alcohol after TBI, treating AUD in people with TBI has the potential to markedly improve outcomes and reduce the likelihood of devastating repeated injuries.

Treatment of Co-Occurring TBI and AUD

There are special considerations for treating co-occurring AUD and TBI. As already mentioned, people who have TBI may be disproportionately vulnerable to negative consequences of alcohol misuse. However, there are unique challenges and opportunities for treatment of AUD among people with TBI. After their injuries, many patients with TBI significantly reduce the amount of alcohol they drink.[21,30] Although a substantial subset (approximately 25%) of these individuals eventually returns to (or surpasses) preinjury drinking levels, this initial period of abstinence has been characterized as a "window of opportunity" for screening and intervention. There is limited, but generally positive, evidence that brief interventional strategies and cognitive-behavioral therapies can be effective in this population.[52]

Although screening and monitoring for AUD are key steps in the management of TBI, many patients, particularly those who do not receive specialized or follow-up care, are not assessed for AUD risk. Moreover, patients with TBI represent a special challenge for treatment of AUD. TBI is a heterogeneous condition, but there are certain brain regions that are more likely to be damaged because of their anatomical location. These regions include the key areas for cognitive control and executive function in the frontal and anterior temporal regions. Thus, it is extremely common after moderate to severe TBI to suffer from cognitive deficits, impaired emotional regulation, and difficulty focusing attention. Therefore, AUD treatment protocols must be tailored to address the specific challenges of this population.

Additionally, people with TBI have high rates of neuropsychiatric comorbidities, including depression, anxiety, and PTSD, all of which can promote alcohol misuse and complicate AUD treatment.[60] Treatment for comorbid psychiatric disorders, particularly addiction, is more challenging in patients with a history of TBI, but the existing evidence indicates that treatments targeting both PTSD and comorbid alcohol dependence produced greater reductions in symptoms for both disorders than treatments for either condition alone.[64]

Moreover, the efficacy of drugs (e.g., disulfiram and naltrexone) approved specifically for treatment of AUD has been minimally investigated in the TBI population.[65] These drugs are not contraindicated for people who have TBI, but medication for this population tends to require careful titration and close monitoring of responses. Also, the elevated risks of substance misuse should be considered when using medication to manage TBI symptoms in this patient population.

The pharmacological treatments for management of TBI fall into two general classifications.[66] In the acute phase after injury, a small number of compounds are administered to manage symptoms and to (attempt to) reduce damage from the initial injury. In the later phases, several psychoactive compounds (e.g., cholinesterase inhibitors, stimulants, and amantadine) are prescribed to modulate cognitive symptoms, fatigue, and insomnia.[66-68] Although little direct evidence indicates that these compounds can increase the likelihood of developing AUD, it is imperative to consider how their potential and efficacy are influenced by alcohol if they are to have appropriate clinical effects.

Mechanisms Linking AUD to TBI

There are a number of potential mechanisms that link TBI to AUD across both cognitive and psychosocial domains. Further, there is mounting evidence that central inflammatory signaling can interact with deficits in neural reward systems,

which may indicate that people with TBI are more vulnerable to developing AUD.

Cognitive and psychosocial links

The incentive motivation theory of drinking predicts that individuals drink alcohol to either enhance positive affect (i.e., directly improve mood or facilitate socialization) or reduce negative affect (i.e., alleviate depression or anxiety).[69] The decision to drink or not drink alcohol, as predicted by this theory, is based on weighing the perceived benefits against the potential costs, which may include legal and occupational issues, hangovers, monetary costs, and social pressures. However, people with TBI often have difficulty weighing the future costs of their actions. For instance, laboratory-based neuropsychological tests demonstrate that people who have frontal lobe injuries consistently have deficits in decision-making, as assessed by their performance in delay discounting and gambling tasks that require judgment about future consequences of immediate actions.[70,71] This pattern of cognitive deficits is superficially similar to what occurs in patients with AUD, and these cognitive deficits are worse in patients with TBI who meet the diagnostic criteria for AUD.[72] Thus, despite future negative consequences, people with TBI may be less likely than those without TBI to decide to not drink.

Neurobiological substrates

Neurobiological links between TBI and AUD remain unspecified, although a potential link has received increased attention in recent years, and new animal models have been developed.[73,74] Injury to the brain often results in affective, cognitive, and psychosocial impairments that can promote alcohol misuse. Moreover, the underlying neurobiological roots of these impairments may also render the brain more vulnerable to developing alcohol dependence.

To investigate the potential relationship between TBI during development and future alcohol use, we developed an animal model in which we administered a mild TBI to mice during juvenile development and allowed the animals to grow into adults.[75] Animals that experienced TBI as juveniles exhibited markedly greater alcohol self-administration as adults, when compared to noninjured animals. The difference in alcohol self-administration between the two groups of animals was independent of changes in sensory function. Also, for the mice that had TBI, the difference was associated with enhanced reward responses to intraperitoneal alcohol. Thus, the injury during juvenile development altered the rewarding properties of alcohol. Moreover, we could block the enhanced drinking behavior that followed TBI by housing the animals in enriched environments, which served as a proxy for sustained cognitive and physical rehabilitation. We have begun to use this model to investigate the neurobiological substrates of alterations in alcohol-related circuitry.

For instance, as already discussed in this article, TBIs are remarkably heterogeneous in etiology, location, and severity, but they do possess some common features.[3] Specifically, virtually all TBI produces acute neuroinflammatory response and persistent alterations in neuroimmune physiology.[76] This is important because alcohol and central inflammatory responses are bidirectionally linked. High doses of alcohol produce a characteristic inflammatory response in the brain, including activation of microglia and upregulation of proinflammatory signaling molecules.[59] Further, this inflammatory response to alcohol is exacerbated in animals with a history of TBI. We recently showed that mice that experienced TBI during juvenile development exhibited exaggerated inflammatory responses, cognitive deficits, and neural degeneration following binge-like alcohol administration in adulthood.[77] Moreover, inflammatory responses in the brain drive alcohol-drinking behavior in animals, and blocking or reducing neuroinflammatory signaling can attenuate alcohol self-administration.[78-80] Thus, we postulate that TBI establishes a state of constant escalation in which it directly induces an inflammatory response and also enhances the neuroinflammatory response to subsequent exposure to alcohol.[73] Future studies need to address whether inhibiting TBI-induced inflammatory responses can also prevent increases in drinking alcohol.

TBI also may produce a state of hypodopaminergia. In clinical populations, imaging data and the widespread use of dopaminergic agents (e.g., methylphenidate and amantadine) for the treatment of TBI-related cognitive issues provide indirect evidence of the hypodopaminergia.[14] Whether the effectiveness of dopaminergic agents in patients with TBI reflects

a true dysregulation of mesocorticolimbic dopamine, or if higher dopaminergic tone is beneficial for cognitive function in survivors of TBI, remains unspecified. However, in animals, TBI produces a biphasic alteration in dopamine signaling characterized by an initial upregulation of dopaminergic synthesis pathways and dopamine release, followed by prolonged suppression.

Neuroinflammatory responses have significant antidopaminergic effects,[81] and blunted dopaminergic release is a major risk factor for the development of AUD.[82] In our juvenile TBI model, injured mice exhibited markedly attenuated dopaminergic signaling in adulthood and altered patterns of neuronal activation in dopaminergic cells.[83] There are many unanswered questions, but injury during periadolescent development in mice seems to persistently alter the development of the dopaminergic system and the response to alcohol in this key reward system. Clearly, there are many other mechanisms beyond neuroinflammation and hypodopaminergia that could underlie greater vulnerability to AUD in people with TBI, and this review is limited in scope.

Future Research Needs

There are many unanswered questions regarding the relationship between TBI and AUD. Most pertinently, we need to determine if TBI exacerbates AUD or increases vulnerability to the development of AUD. We also need to ascertain how underlying neural mechanisms affect TBI and AUD. In particular, what are the roles of chronic neuroinflammatory signaling, impairments in reward processing, and cognitive issues in mediating susceptibility to AUD? We know that many people with TBI meet the diagnostic criteria for AUD and continue to drink alcohol after their injuries. Further, we know this pattern of behavior is associated with varied, but serious, negative consequences. Thus, future research needs to address the best ways to screen and treat people with TBI to minimize the harm associated with drinking alcohol after injury.

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Common Biological Mechanisms of Alcohol Use Disorder and Post-Traumatic Stress Disorder

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Post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) are highly comorbid. Although recent clinical studies provide some understanding of biological and subsequent behavioral changes that define each of these disorders, the neurobiological basis of interactions between PTSD and AUD has not been well-understood. In this review, we summarize the relevant animal models that parallel the human conditions, as well as the clinical findings in these disorders, to delineate key gaps in our knowledge and to provide potential clinical strategies for alleviating the comorbid conditions.

KEY WORDS: **addiction; animal models; depression; neural circuitry; post-traumatic stress disorder (PTSD); stress; trauma**

Alcohol use disorder (AUD) is one of the most common co-occurring disorders among individuals diagnosed with post-traumatic stress disorder (PTSD).[1] Many people who have PTSD use alcohol in an attempt to ameliorate debilitating symptoms such as anxiety and hyperarousal. Clinical and epidemiological studies have consistently reported that PTSD is associated with a threefold higher risk for developing AUD, and for individuals who have PTSD, the lifetime prevalence of AUD has been estimated at 40%.[2] The severity of PTSD symptoms is positively related to the level of alcohol use, and it also predicts alcohol craving in response to trauma- and alcohol-related cues. Despite the high rates of comorbidity, there is a substantial gap in understanding how traumatic experience leads to transition from initially controlled alcohol consumption (reward phase) to the development of alcohol-seeking and dependence (negative reinforcement phase). This review summarizes clinical observations and highlights findings from preclinical animal models, and focuses particularly on the alterations and dysfunctions in neural circuitry and stress hormone systems that may underlie enhanced vulnerability to AUD in context of PTSD (Figure 1).

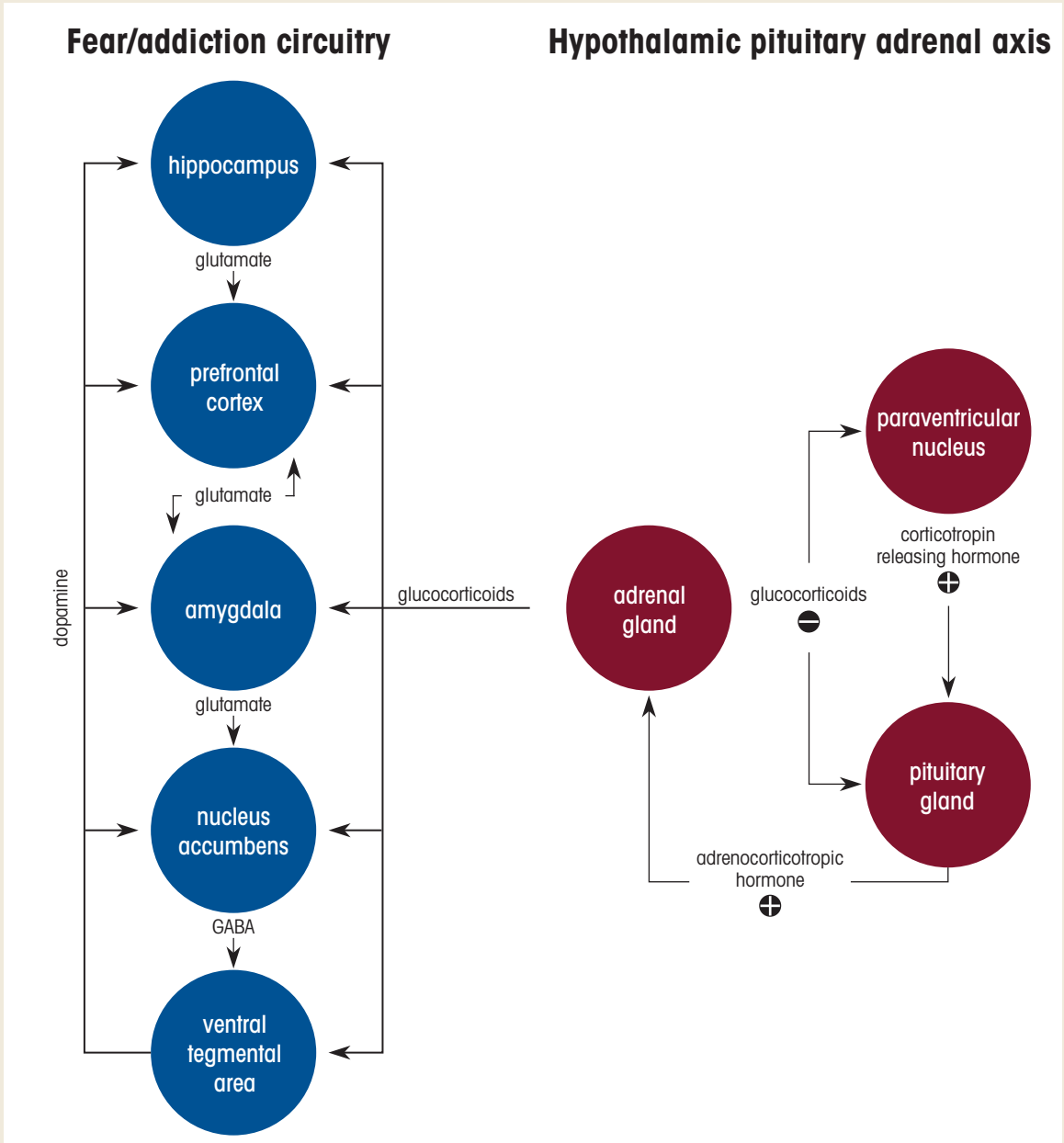

Figure 1 Interactions between the fear/addiction neural circuitry and the hypothalamic pituitary adrenal (HPA) axis. The fear/addiction circuitry includes the hippocampus, prefrontal cortex, amygdala, nucleus accumbens, and ventral tegmental area. The prefrontal cortex mutually connects with the amygdala, and the amygdala projects to the nucleus accumbens via its glutamatergic innervations. All these areas receive projections from dopamine neurons in the ventral tegmental area. The major components of the HPA axis include the paraventricular nucleus of the hypothalamus and the pituitary and adrenal glands. Corticotropin releasing hormone from the paraventricular nucleus stimulates adrenocorticotropic hormone (ACTH) release from the anterior pituitary into the bloodstream, then ACTH induces glucocorticoid release from the adrenal gland. Glucocorticoids mediate negative feedback in the HPA axis to reduce the stress response. Glucocorticoids also affect the fear/addiction circuitry via the glucocorticoid receptors, which triggers molecular, cellular, and physiological changes, including epigenetic alterations. *Note:* GABA, gamma-aminobutyric acid.

Preclinical Models of PTSD and AUD

Animal model approaches

There are several procedures commonly used to create animal models of stress or PTSD and to employ stress components that are known to lead to enhanced risk for AUD.[3] Many procedures are simple, easy to implement, and effective at inducing a broad departure from endocrinological, physiological, and neurobiological homeostasis.[4] Also, both acute and chronic stressors can lead to physical and psychiatric pathology. First, we briefly describe a range of stress-related approaches to modeling the phenotypes of PTSD and AUD. Then, we review supporting studies in more detail, examining common biological components of both disorders.

Widely used physical stressors include exposure to immobilization, restraint, cold-water swimming, electric footshocks, and noxious stimuli.[4] Immobilization or restraint stress commonly is produced by confining a naïve animal inside a bag or tube. Also, relevant naturalistic or ethological stressors have been used to trigger stress states.[4] Models of psychological stress include exposure to predator odor; an elevated platform; or a bright, open area; whereas models of social stress include social isolation, maternal deprivation, and social defeat. In some studies, more than one stressor is applied concomitantly to test the generality of a hypothesized mechanism or to enhance the intensity of desired responses.

Alcohol behaviors include various responses and changes elicited by alcohol exposure and withdrawal. Examples of these behaviors are alcohol craving, compulsive alcohol-seeking, excessive alcohol intake, alcohol dependence, and relapse. In this review, we survey the recent progress in animal modeling for two main aspects of AUD-related alcohol behaviors—alcohol consumption and alcohol-seeking. In general, experiments designed to investigate the effects of stress and alcohol behaviors can be divided into three categories. In the first category, alcohol-naïve animals experience stress, then alcohol is introduced concurrently or after an incubation period.[5-7] In the second category, animals are familiarized to alcohol or to drinking alcohol before stress is introduced.[8] In the third category, animals develop alcohol behaviors, subsequently extinguish those behaviors, and then stress is introduced during a development, extinction, or reinstatement period.[9] In these experimental designs, alcohol behaviors are generally monitored through preference ratios and by measuring intake. Typically, animals have free access to water or an alcohol solution, and alcohol preference and intake are determined by the amount of liquid consumed and the number of approaches.

A considerable body of evidence suggests that stress triggers negative affective states and subsequent adaptive changes that lead to the development of AUD, so many animal models for AUD have focused on creating a condition in which a stress procedure precedes alcohol exposure (or re-exposure).[3] Notably, however, it also has been suggested that excessive drinking is a risk factor for developing anxiety disorders such as PTSD. There are several reasons this may be the case. One possibility is that in cortical regulatory areas such as the medial prefrontal cortex (mPFC), impairments from excessive drinking are similar to impairments from repeated stress. For example, in a 2012 study of mice, Holmes and colleagues examined the effects of chronic alcohol exposure on the prefrontal cortex (PFC) and its capacity to mediate fear extinction.[8] Fear extinction is a reduction in the frequency or intensity of a conditioned fear response (e.g., freezing) after repeated presentation of a conditioned stimulus (e.g., a sound) in the absence of the unconditioned aversive stimulus (e.g., a footshock). Holmes and colleagues found that mice intermittently exposed to continuous vaporized alcohol had significant remodeling of mPFC neurons and demonstrated impaired fear extinction.[8]

Using a combination of these preclinical models and molecular, genetic, and pharmacologic manipulation approaches, recent investigations have made great strides in delineating the neurobiological processes underlying stress-induced escalated alcohol intake or alcohol-seeking behavior. Next, we summarize some details of these models and their relevance to both disorders, as well as to comorbid PTSD and AUD.

Restraint or immobilization stress

Restraining rodents in small tubes or on a platform in an acute or chronic manner leads to increased

manifestations of anxiety and changes in neuronal morphology within brain regions that mediate fear and anxiety.[10,11] In previous studies, acute immobilization stress in mice significantly elevated hypothalamic pituitary adrenal (HPA) axis activity, resulting in impaired fear extinction and extinction retention following Pavlovian fear conditioning.[12,13] Furthermore, exposure to this stressor led to impaired long-term declarative memory and enhanced anxietylike behavior.[14]

Because of the practical simplicity of restraint-related procedures, numerous studies have employed them to elucidate the relationship between stress and alcohol consumption. However, the results are not conclusive. In some cases the stressor significantly increased alcohol intake, whereas in others alcohol consumption decreased or did not change.[15,16] Therefore, although researchers have speculated about many factors, such as time, individual differences, and stress-induced long-term sensitization or desensitization of the HPA axis,[17] there appears to be no clear primary determinant on the outcome in those studies.

Social stress

Social isolation, such as maternal deprivation, is a demonstrated risk factor for alcohol consumption during adolescence and adulthood, particularly in male rats.[18] In one study, when rat pups were separated from their mothers for 6 hours per day for 20 days, they exhibited increased ethanol consumption during their adolescence, compared with rat pups that had only 15 minutes of deprivation per day. In a similar study, rats (male and female) that experienced a single, 24-hour maternal deprivation on postnatal day 9 and subsequent exposure to restraint stress showed higher ethanol intake than animals that experienced only a single maternal deprivation.[19] Furthermore, isolation stress during adolescence seemed to similarly increase alcohol consumption. For example, rats housed individually during adolescence exhibited increased ethanol intake and ethanol preference during adulthood.[20] Moreover, when an intermittent procedure was used to offer these rats alcohol, they drank significantly more ethanol solution and obtained higher blood ethanol levels than rats that received a continuous procedure. In addition, when induced by chronic early life stress, the increase in ethanol consumption lasted for at least 8 weeks.[21] Notably, the stressed rats displayed a significant deficit in fear extinction but not in fear memory acquisition.

Also, several studies have shown through self-administration and place-conditioning paradigms that exposure to social defeat stress induced escalation of alcohol consumption as well as reinstatement of alcohol-seeking behavior after extinction.[22] Procedures for invoking social stress can be divided into acute versus repeated, or agonistic encounters in a neutral environment versus resident or intruder settings. In these stress paradigms, the observation of escalated alcohol intake is related to when the stress experience occurred. The animals showed no significant change in alcohol consumption immediately after stress, but they showed an increase 2 hours after stress.[22]

More recent studies with mice demonstrated that a 10-day social defeat stress experience increased ethanol drinking and preference for at least 20 days after the defeat.[6,7] Elevated alcohol consumption was correlated with plasma corticosterone levels and was modulated by the signaling pathway of corticotropin releasing hormone receptor 1 (*CRHR1*) in the ventral tegmental area (VTA) and by dopamine within the nucleus accumbens. Chronic social defeat in rats and mice is well-known for inducing some core PTSD symptoms, such as increased social avoidance[23] and anxiety,[22] as well as enhanced fear memory acquisition.[23]

Predator-based stress

In rodents, exposure to a natural predator has been shown to provoke high levels of intense fear and stress, followed by long-lasting endocrine and behavioral responses. Typically, the rodents are exposed very briefly (5 to 10 minutes) to a predator or to predator odorants, such as predator urine, which leads to elevation of long-lasting anxietylike behavior.[24] Specifically, rats exposed to chronic social instability in conjunction with cat odor showed reduced basal glucocorticoid levels, increased glucocorticoid suppression following dexamethasone administration, heightened anxiety, and enhanced fear memory.[25] These results mimic common endocrine and behavioral measures found in humans with PTSD. Another study demonstrated that rats with higher stress reactivity

to predator urine exhibited more alcohol drinking than rats with lower stress reactivity.[5]

Genetic differences

It has been well-reported that background strain differences can confound stressor reactivity measures and alcohol-related behaviors in the same manner demonstrated for other behavioral measurements, including learning and memory performance, aggression, and emotionality. For example, a phenotypic survey study comparing fear extinction in a panel of inbred mouse strains revealed fear extinction impairment in the 129/SvImJ strain due to a failure in the engagement of corticolimbic extinction circuitry, despite the strain's normal fear conditioning and nociception.[26] A similar study showed that chronic exposure to swim stress resulted in a significant decrease in ethanol consumption in mouse strains DBA/2J and BALB/cByJ but not in strain C57Bl/6J, although stress increased sensitivity to the sedative/hypnotic effects of ethanol in all three strains.[27]

Neurobiological Circuits

Neuroimaging studies have suggested that stress-induced alcohol behaviors may relate to convergent or divergent changes in multiple brain areas. However, to provide a framework for identifying alterations in neural circuitry, we will focus on a few brain areas well-associated with processing fear, anxiety, stress, and rewards. These areas include the amygdala, PFC, hippocampus, and VTA.

Amygdala

The amygdala is well-known for its role in physiological and behavioral responses to fear, stress, and substance misuse.[5,28,29] During fear learning, the amygdala receives multisensory information from the cerebral cortex and thalamus and projects to brain regions that produce behavioral and physiological fear responses.[28] During fear extinction and fear extinction recall, the mPFC and hippocampus regulate the amygdala from the top down through rich, mutual connections between these areas to modulate previously conditioned fear. Furthermore, severe stress facilitates fear and anxietylike behavior via amygdala-dependent anatomical and physiological changes at synaptic, cellular, and network levels.[4,28,29] Neuroimaging studies of healthy humans have shown that increased amygdala activity was evoked by fearful cues and during fear conditioning.[30] In other studies, combat veterans with PTSD who were exposed to fearful faces exhibited higher levels of amygdala activation than healthy individuals, and they also exhibited hyperreactivity in the presence of trauma-related stimuli.[31,32]

In a 2014 study, Garfinkel and colleagues examined amygdala activity in individuals with PTSD.[33] The researchers used conditioning to generate a fear response to a conditioned stimulus of a colored light (the dangerous context). Later, in a different (safe) context, participants were conditioned to extinguish that fear response. The individuals with PTSD exhibited an increase in amygdala activity when reintroduced to the conditioned stimulus in the safe context, indicating impaired fear extinction. However, in the same study, individuals with PTSD demonstrated low amygdala activity when the extinct conditioned stimulus was reintroduced in the original dangerous context to elicit a fear response (i.e., fear renewal). The low amygdala activity could indicate that these individuals have impaired fear renewal. These findings suggest that individuals with PTSD have a globally diminished capacity to use contextual information to modulate fear expression.

In addition to functional changes, structural changes in the amygdala have been reported in individuals who have PTSD and a history of early life stress. Notably, smaller amygdala and hippocampus volumes have been found in children exposed to different forms of early life stress and have been associated with greater cumulative stress exposure and behavioral problems.[34] Interestingly, in men who had alcohol dependence, amygdala volume reduction was associated with increased alcohol craving and intake.[35] Furthermore, it has also been demonstrated that alcohol cues triggered amygdala reactivation in men with alcohol dependence alone,[35] as well as in individuals who had PTSD and AUD.[31] However, the neuroimaging data generated by functional magnetic resonance imaging and positron emission tomography do not yet provide the resolution to reliably differentiate amygdala nuclei.

Studies with animal models greatly help extend understanding of the structures and functions

of the amygdala in anxiety and fear memory, because the gross anatomy, connectivity, and cellular composition of amygdala nuclei are well-conserved across species.[28] The amygdala comprises multiple interconnected nuclei that can be classified largely into two groups: cortexlike and striatumlike structures. The cortexlike structure includes the basolateral complex, consisting of the lateral, basolateral, and basomedial amygdala. The striatumlike structure consists of the central nucleus of the amygdala (CeA), which has lateral and medial subdivisions and intercalated cell clusters. During fear conditioning, output activity in the medial division of the CeA is enhanced by excitatory signals originating directly from the lateral amygdala and indirectly through the basolateral amygdala. The output also is modulated by reciprocal connections between the basolateral amygdala and the prelimbic area of the PFC. In contrast, during fear extinction, neural activity in the lateral and basolateral amygdala is reduced, and the infralimbic area of the PFC participates in suppression of fear through the basolateral amygdala and the intercalated cells.

Recent studies suggest functional and molecular heterogeneity for the cell types and projections within some of the amygdala subnuclei. For example, in one of our studies, we found that tachykinin receptor 2 (*TACR2*)-expressing neurons in the medial division of the CeA were involved in fear consolidation.[36] In another study, researchers found that protein kinase C delta (*PRKCD*) expression in the lateral division of the CeA provided inhibitory regulation in the medial division of the CeA, reducing fear expression.[37] Similarly, through optogenetic manipulations, we demonstrated that Thy-1 cell surface antigen (*THY1*)-expressing neurons in the basolateral amygdala were involved in fear extinction and fear extinction recall.[38,39]

Because a generalized fear response is considered a hallmark of anxiety, researchers have examined intra-amygdala circuits and long-range projections and demonstrated that microcircuits in the amygdala play a role in anxiety. In one study, increased tonic firing of output neurons in the medial division of the CeA activated by neurons in the lateral division of the CeA was required for fear responses to the conditioned stimulus and to an unconditioned stimulus.[40] These findings suggest that tonic activity within CeA fear circuits may be an underlying neuronal substrate for anxiety. Similarly, in the lateral amygdala, activity in distinct neuronal populations also seems to be necessary for fear generalization. One study reported that in rats that exhibited generalized fear, cells in the lateral amygdala responded to a conditioned stimulus that was not paired with an unconditioned stimulus.[41]

Because alcohol-seeking in humans has long been considered to be motivated by the desire to reduce stress and anxiety, the amygdala has been linked to behavior associated with alcohol misuse. In particular, the gamma-aminobutyric acid (GABA) neurotransmitter system in the CeA has been implicated in mediating behavior associated with acute and chronic alcohol consumption. In one study, rat brain slices exposed to an acute superfusion of ethanol increased presynaptic GABA release and enhanced postsynaptic GABA receptor function in CeA neurons.[42] The same researchers also demonstrated that chronic ethanol exposure promoted increased basal GABA release without presynaptic effects.[43] Furthermore, stereotactic injection of gabapentin, an anticonvulsant GABA analog, attenuated elevated operant ethanol responses in ethanol-dependent rats.[43] Studies with transgenic mice showed that ethanol enhanced the activity of CRHR1 receptors in the CeA, implicating potential cell type–specific interactions between the stress corticotropin releasing hormone (CRH) signaling pathway and alcohol consumption and dependence.[44] Consistent with this idea, studies have shown that rats that displayed persistent avoidance of a predator odor–paired context consumed more alcohol and exhibited compulsivelike responding for alcohol,[5] and they expressed hyperalgesia via the CRH signaling pathway in the CeA.[45]

PFC

The PFC, a large and complex brain region that is greatly expanded in nonhuman primates and humans, is topographically organized and has anatomically distinct subfields, roughly divided into dorsolateral, ventromedial, and orbital regions. These subfields are believed to be involved in various cognitive and emotional functions. For example, the dorsolateral regions of the PFC provide top-down regulation of attention, thought, and action and have extensive connections with sensory and motor cortices.[46] In contrast, the ventromedial regions of the PFC regulate emotional responses

and have vast connections with various subcortical structures, such as the amygdala, nucleus accumbens, and hypothalamus.[47] The PFC also has direct and indirect interactions with the monoamine system, including noradrenergic projections from the locus coeruleus and dopaminergic inputs from the substantia nigra and VTA. The PFC is sensitive to the detrimental effects of stress exposure, as even mild uncontrolled acute stress can cause a rapid and dramatic loss of cognitive abilities, and more prolonged stress exposure causes anatomical changes in the PFC. All of these PFC pathways are critically involved in appetitive behavior, as occurs with AUD, and in emotion regulation, which is disrupted during fear processing, as occurs with PTSD.

Given the mutual connectivity between the PFC and amygdala, it has been suggested that the fortified emotional memory traces in individuals with PTSD may be a product of imbalanced interactions between the two brain areas. The PFC seems to exert an inhibitory response on the amygdala, which is a central node for emotional reactivity. In neuroimaging studies, participants with PTSD showed decreased prefrontal blood flow,[48,49] and a study that used trauma reminders to provoke symptoms in patients with PTSD reported reduced activation in the ventromedial PFC.[50] This decreased PFC activity is often accompanied by increased amygdala activity,[49,51] suggesting there may be a failure of top-down cortical inhibition on the reactivation of memory traces associated with trauma-related thoughts and feelings.

The failure of top-down cortical inhibition may also relate to functional mechanisms associated with stress-related alcohol craving and relapse. Alcohol-related dysfunction in the PFC affects higher order executive function, including response inhibition and decision-making. Alcohol-related neuroadaptations in the prefrontal networks, including in the corticostriatal motivation pathways,[52] could also promote increased relapse risk and craving for alcohol consumption. In support of these ideas, researchers have used individually calibrated, script-driven, guided-imagery procedures and neuroimaging to identify neural responses to stress and alcohol context cues.[53,54] These studies demonstrated that, in healthy individuals, stress and alcohol cue exposure induced overlapping neural responses, with increased activation of the corticolimbic striatal circuit, encompassing the mPFC, orbitofrontal cortex, and anterior cingulate cortex. Healthy men displayed greater stress-induced activations throughout the prefrontal areas than healthy women, whereas women showed greater alcohol cue–related activity in the superior and middle frontal gyrus than men.[53] These findings suggest that differential neural responses in these cortical areas may contribute to the sex differences found in stress-related coping and in vulnerabilities to stress-induced alcohol consumption and alcohol-seeking.

A follow-up study with a similar approach showed that individuals with AUD, when compared with control subjects, had less neural activity in the ventromedial PFC and anterior cingulate cortex when exposed to an alcohol-enticing or stressful stimulus.[54] These same participants showed increased activity in the ventromedial PFC and anterior cingulate cortex during exposure to relaxing cues. These neuroimaging studies indicate that disrupted functions in the PFC, as well as in motivation-reward brain regions, may be neural mechanisms underlying alcohol craving and relapse.

Although it has been difficult to determine exactly analogous rodent and human brain regions, it is generally accepted that rodents have a PFC equivalent.[55] Based on examination of rodent cellular structure, lamination, and projection patterns, findings suggest there are clear distinctions between the dorsal (precentral and anterior cingulate) and ventral (prelimbic, infralimbic, and medial orbital) subdivisions of the mPFC.[47] The rodent dorsal PFC, similar to the primate PFC, is implicated in memory for motor responses, including the temporal processing of information and response selection.[56] The ventral PFC is involved in emotional responses, such as anxiety, and in the expression and extinction of conditioned fear memory.[57,58]

Hippocampus

The hippocampus is defined by its characteristic trisynaptic circuit and is well-known for its crucial roles in spatial navigation and episodic memory (i.e., recall of events within the spatial and temporal context in which they occurred).[59] Dysfunctions of the hippocampus lead to not only memory deficits, but also anxiety, depression, epilepsy, and schizophrenia, suggesting that the hippocampus contributes to attention, arousal, and emotional

states, including stress.[60] Stress produces intense and long-lasting memories that can be a source of serious distress, but prolonged stress seems to impair performance on hippocampus-dependent memory tasks. For example, individuals diagnosed with PTSD and healthy individuals injected with cortisol (a human glucocorticoid) have been shown to be impaired in various verbal recall tests.[61] In addition, clinical and preclinical studies have shown that stress changes synaptic plasticity and firing properties of hippocampus neurons, induces morphological atrophy, suppresses neuronal proliferation, and reduces hippocampal volume.[61] These wide-ranging changes appear to be mediated by stress hormones. Glucocorticoids act, in part, via negative feedback of the HPA axis through the hippocampus, which is densely concentrated with glucocorticoid receptors. Similarly, rodent studies have shown that exposure to stress or high doses of corticosterone (a rodent glucocorticoid) produces deficits in hippocampus-dependent spatial memory tasks.[60]

Neuroimaging studies have demonstrated that acute alcohol exposure affects the hippocampal function of contextual or episodic memory encoding.[62] In addition, chronic alcohol misuse seems to cause a reduction in hippocampal volume and activity.[63,64] In animal studies, alcohol exposure during fetal or adolescent development has been shown to induce reductions in hippocampal neurogenesis.[65,66] In addition, chronic alcohol exposure has been shown to disrupt adult hippocampal neurogenesis, alter connectivity of new neurons, and result in behavioral deficits, as demonstrated through the hippocampus-dependent novel-object recognition task and Y-maze test.[67]

VTA and dopamine regulation

The VTA is in the midbrain, situated adjacent to the substantia nigra, and it is primarily characterized by its dopaminergic neurons, which project to limbic and cortical areas via the mesolimbic and mesocortical pathways, respectively. Electrophysiological studies in monkeys demonstrated that rewards and reward-predicting cues elicited strong phasic firing of midbrain dopamine neurons.[68] Functional magnetic resonance imaging studies in humans have reported that increased midbrain activation occurred during anticipation of pleasant tastes[69] and monetary gains,[70] as well as for reward-predicting cues.[71] Because VTA dopamine neurons project densely to the nucleus accumbens in the ventral striatum via the mesolimbic pathway, these brain areas have been implicated as major areas for processing natural rewards, reinforcement, and drugs of abuse.[72]

Studies using pharmacological perturbation and biochemical measurements have provided strong evidence for the reinforcement role of alcohol via the mesolimbic dopamine system. In a study with rats, systemic injection of dopamine receptor antagonists decreased responding for alcohol in a free-choice task, but the injection did not affect responses for water.[73] Furthermore, in a study of nondependent rats, alcohol self-administration increased extracellular levels of dopamine in the nucleus accumbens.[74] Such increases occurred during and also before the self-administration, indicating the motivational properties of cues associated with alcohol. Similar results have been shown in dopamine neurons of monkeys responding to reward cues.[68]

Acute exposure to different forms of stress reportedly increases dopamine release in the nucleus accumbens,[75] whereas long-term, repeated exposure to different stressors decreases basal dopamine output in the nucleus accumbens.[76] If the base level of dopamine has been reduced by stress, the phasic dopamine release induced by alcohol may have an amplified effect. This amplified dopamine effect may further enhance the reward-learning process, consequently leading to increases in alcohol consumption and preference.

Stress-induced alcohol preference and alcohol consumption seem to be due to alterations in both excitatory and inhibitory circuits within the VTA. A 2013 study in rats demonstrated that social isolation stress enhanced the acquisition of memories for alcohol-associated environmental cues.[77] The learning processes were facilitated by long-term potentiation of *N*-methyl-D-aspartate (NMDA) receptor–mediated excitatory transmission in the VTA, and the facilitation could not be reversed by resocialization. In contrast, Ostroumov and colleagues showed that stress promoted alcohol use through actions on inhibitory GABA signaling in the VTA.[78] Rats that underwent acute restraint stress 15 hours before introduction to ethanol self-administered considerably more ethanol than controls, and this increase in alcohol consumption

lasted for more than 7 days. Electrophysiological recordings in the same study revealed that stress blunted the ethanol-induced increase in the firing rate of VTA dopamine neurons, which was restored by application of a $GABA_A$ receptor antagonist. The stress also increased the concentration of intracellular chloride ions in VTA GABA neurons and seemed to alter the chloride gradient of GABA neurons such that, paradoxically, GABA excited these cells.

VTA dysfunction is clearly relevant to AUD. However, in PTSD, both the anhedonic component and the dopamine regulation of fear extinction may represent neuroanatomical VTA dysfunction, which may contribute to AUD and PTSD comorbidity.

Stress Axis Function

HPA axis

The HPA axis is the main neuroendocrine response system to stress.[61] The activation of this system is characterized by adrenal gland synthesis and release of steroids known as glucocorticoids, such as cortisol in humans and corticosterone in rodents, triggered by the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH release into the general circulation is controlled by the secretion of CRH from the paraventricular nucleus of the hypothalamus to the anterior pituitary gland via the portal blood vessels.

Glucocorticoids act on the brain through two main receptors: type I, the mineralocorticoid receptor (MR), and type II, the glucocorticoid receptor (GR). These are nuclear receptors working as transcription factors. They modulate targeted gene expression by binding to DNA or by interfering with the activity of other transcription factors.[61] Notably, the MR has a 10-fold higher binding affinity for glucocorticoids than the GR. This differential binding affinity is assumed to create a two-tier system with negative feedback.[79] Due to their high affinity, MRs are bounded by glucocorticoids and appear to be in a constant activated state under any physiological condition. In contrast, GRs with low binding affinity are occupied only after a significant rise of glucocorticoids. These GRs play a role in exerting negative feedback on enhanced HPA axis activity and in stress-related adaptation.[79]

As part of homeostatic processes, the actions of the HPA axis are tightly regulated to ensure that the body can optimally face stress challenges, adapt to environmental stimuli, and return to a normal state. Dysfunctions in the HPA axis frequently have been found in humans diagnosed with PTSD or AUD, so comorbidity may stem from an overlapping neurobiological mechanism. However, the details of this mechanism as a possible link between these disorders are not yet well-understood. In this section we describe recent findings on PTSD or AUD in humans and animals and how these conditions relate to the role of the HPA axis in comorbid high-stress reactivity and enhanced alcohol intake.

Stress hormones and PTSD

Neuroendocrine studies have shown profound alterations in the HPA axis in individuals with PTSD. In particular, it has been well-documented that reduced baseline cortisol levels, in addition to enhanced cortisol suppression to a low-dose dexamethasone challenge, are present in some individuals with PTSD.[80] These individuals also displayed augmented cortisol feedback inhibition of ACTH secretion at the level of the pituitary and a blunted ACTH response to CRH. Furthermore, because studies have consistently shown that individuals with PTSD have glucocorticoid receptor hypersensitivity, lower cortisol levels in plasma could be due to homeostatic feedback.

Glucocorticoids readily cross the blood-brain barrier, exert negative feedback at the HPA axis, and consequently reduce CRH and ACTH secretion (Figure 1). They also bind to MRs and GRs throughout the brain, including in the amygdala, hippocampus, PFC, nucleus accumbens, and septum, where they influence signaling pathways and synaptic plasticity. It has been hypothesized that different anatomical populations of GRs in the brain have unique functions in modulating plasma glucocorticoid levels. For example, in one study, application of corticosterone to the hippocampus inhibited HPA axis activation in male rats.[81] However, in a different study, hormonal stimulation to the amygdala in rats increased plasma corticosterone and increased CRH expression in the CeA.[82] Recent studies that used conditional knockout mouse models demonstrated that the ablation of GRs in glutamatergic, but not in

GABAergic, neurons induced hyperreactivity in the HPA axis and reduced fear- and anxiety-related behavior.[83] Furthermore, viral-mediated deletion of GRs indicated that within the basolateral amygdala glutamatergic circuits, GRs played a role in fear expression but not in anxiety. The findings suggest that fear-related behavior is modulated by GR-signaling pathways in the basolateral amygdala, whereas pathological anxiety may result from altered GR signaling in excitatory circuits in several brain areas, including the bed nucleus of the stria terminalis—which is also potentially involved in AUD and PTSD.

CRH and its receptors are expressed not only in stress-responsive areas, but also in areas of the fear- and threat-processing circuits, including in the basolateral amygdala and CeA. It has been shown that infusion of CRH or CRH binding protein into the basolateral amygdala prior to fear extinction impairs extinction recall without affecting extinction acquisition.[84] In contrast, a CRH receptor antagonist improved extinction recall. A study that used a conditional knockout mouse model demonstrated similar results.[85] Deletion of the $alpha_1$ subunit of the $GABA_A$ receptor in CRH-expressing amygdala neurons resulted in increased CRH expression in the amygdala. Consequently, anxiety behavior increased, and extinction of conditioned fear was impaired, which coincided with increased corticosterone levels in plasma.

Stress hormones and alcohol intake

Many individuals with AUD show altered HPA axis function, raising the strong possibility that HPA axis dysfunction contributes to the development of AUD. Several studies with animal models also demonstrated that the HPA axis plays a direct role in the control of alcohol drinking. For instance, administration of corticosterone into the body or brain of rats increased their voluntary alcohol drinking, whereas administration of a corticosterone synthesis inhibitor or the removal of the adrenal glands caused decreased alcohol intake.[86,87] Furthermore, a recent study demonstrated that attenuation of GR signaling reduced compulsivelike alcohol intake in alcohol-dependent rats and reduced both excessive drinking and alcohol craving in recently abstinent individuals with AUD.[88]

Given that alcohol increases dopamine release in the nucleus accumbens in animals[89] and humans,[90] glucocorticoids may be involved in voluntary alcohol consumption via direct action on mesocorticolimbic reward systems where GRs are abundantly expressed. A study that used a mouse model demonstrated that selective ablation of GRs in dopaminergic neurons in the brain, or of dopamine receptor D1-expressing medium spiny neurons in the striatum, highly reduced the firing rate of dopamine neurons.[91] In the same study, mice with GR ablation in D1-expressing neurons, not in dopaminergic neurons, displayed decreased self-administration of cocaine. These findings suggest that GRs act on the postsynaptic neurons of the dopaminergic system via negative feedback from the nucleus accumbens to the VTA to increase the propensity to self-administer drugs.

In addition to the role of MRs in glucocorticoid regulation, aldosterone and MRs are the principal modulators of blood pressure and extracellular volume homeostasis via renal sodium reabsorption and potassium excretion. Although MRs are expressed in various brain areas, including in the amygdala and hippocampus, their role in stress modulation and alcohol consumption historically has received less attention. Nevertheless, recent studies with rodents, nonhuman primates, and humans have implicated the importance of the aldosterone and MR pathway in alcohol drinking and in alcohol-seeking behavior.[92] Since MRs are also abundantly expressed in the dopaminergic system, future studies using conditional knockout mouse models are needed to determine whether these receptors contribute to alcohol intake and dependence in a manner specific to cell types or brain areas.

CRH and its receptors are also involved in alcohol behavior. In a free-choice paradigm with water and increasing concentrations of alcohol, mice lacking functional CRHR1 receptors increased alcohol intake after repeated episodes of social defeat stress.[93] Notably, these mutant mice did not increase alcohol intake during or immediately after stress, but they did significantly increase intake 3 weeks later. Furthermore, this increased alcohol intake persisted at 6 months after the stress exposure. These findings suggest that the stress response in the HPA axis may require some time for adaptation to concurrent alcohol and stress exposure.

Alcohol-induced stress hormone response

A large body of data suggests that alcohol is a robust activator of the HPA axis. As an example, in one study, plasma glucocorticoids in humans increased during acute and chronic alcohol consumption and during the initial phase of the alcohol withdrawal period.[94] In another study, peripheral injection of alcohol into rats stimulated HPA axis activity, including activating the hypothalamic paraventricular nucleus, CRH release, and ACTH release.[95]

Other neuropeptide systems associated with stress and alcohol

In addition to CRH, numerous neuropeptides have been shown in various animal models to be affected by stress or to be involved in the stress response. Studies on postmortem brain samples showed that other neuropeptides and their receptors could be suitable targets for PTSD and AUD treatments. These neuropeptides include substance P, neuropeptide Y, vasopressin, and pituitary adenylate cyclase–activating polypeptide. Progress in identifying their roles in stress and alcohol consumption has been facilitated by recent preclinical investigations, but we summarize the findings related to only two of those neuropeptides.

Substance P, with its preferred neurokinin 1 (NK1) receptor, is highly expressed in the amygdala and nucleus accumbens. Stressors induce substance P release in the amygdala, and pharmacologic blockade of NK1 receptors inhibits amygdala-associated behavioral responses in rodents.[96] Mice genetically deficient in NK1 receptors have displayed decreased voluntary alcohol consumption and a loss of conditioned place preference for opiates.[97,98] Furthermore, in a study of recently detoxified patients with AUD, treatment with an NK1 receptor antagonist suppressed spontaneous alcohol cravings and blunted cravings induced by a challenge procedure.[97]

Neuropeptide Y is well-known for opposing effects of CRH, reducing stress and anxiety, and decreasing alcohol intake in rodents. Both neuropeptides and their receptors are abundant in the amygdala and extended amygdala, including in the bed nucleus of the stria terminalis. A recent study showed that neuropeptide Y suppressed binge drinking in mice by inhibiting the activity of CRH neurons through a neuropeptide Y_1 receptor–mediated G_i signaling pathway that enhances the ability of GABA to generate inhibitory currents postsynaptically.[99] Chemogenetic activation of CRH neurons in the bed nucleus of the stria terminalis blocked the inhibitory effects of Y_1 receptor activation on binge drinking. The same study demonstrated that chronic alcohol drinking led to persistent alterations in neuropeptide Y_1 receptor function and suggested that shifts in the balance between neuropeptide Y and CRH might change an individual's vulnerability to binge drinking cycles. Moreover, medications that alter this balance could be a good approach for treating binge drinking.

Sex-Dependent Differences

Awareness is increasing regarding the crucial roles that neuronal circuits and hormones play in fear and reward processing differences between men and women. For example, researchers have reported that women suffer from anxiety and PTSD more than men,[100] and that women use alcohol and opioids more frequently than men to handle anxiety.[53] Although research on sex-related differences in comorbid PTSD and AUD is still in its infancy, recent clinical and preclinical studies have started disentangling the neurobiological mechanisms that may place men and women at different risk for the development of each disorder. For example, upon stress cue exposure, men display greater activation in the PFC, amygdala, and hippocampus than women, whereas women showed greater alcohol cue–related activity in brain regions associated with high-level cognitive processing.[53] Furthermore, several studies in rodents have shown sex-related differences in neuronal morphology and in sex-hormone receptor expression in fear circuits, including in the PFC.[101] These sex-related anatomical and molecular differences contribute to disparate functionality in the fear circuits. For example, in a rat study, researchers found that PFC function was important for fear extinction recall in males, but it was critical to fear extinction in females.[102] Similarly, sex-related differences have been detected in the VTA dopaminergic system, and sex hormones have been implicated in differential responsiveness to drugs of abuse.[103]

Conclusions and Future Research Needs

Epidemiological studies suggest that the diagnosis of PTSD represents a major risk factor for the development of AUD, as PTSD symptoms drive excessive alcohol consumption, and AUD worsens PTSD symptoms. Findings from the studies discussed in this article show that a vast array of neurobiological and neuroendocrine changes occur in fear/anxiety and reward/addiction circuitry, as well as in the HPA axis. Analogous changes that occur in overlapping brain areas and high rates of AUD and PTSD comorbidity suggest that these disorders share a common neurobiological etiology.

It has been extremely difficult to systematically delineate the neural basis of comorbidity. Comorbidity may be due to a conjunction of independent risk factors, shared risk factors from two disorders, or a multiform expression of one of the disorders. In this review, we focused on the comorbidity in a context in which one disorder causes the other through dysfunctions in shared neural circuitry. Since the activity of a brain area interacts with and affects other brain areas via mutually connected pathways, investigating comorbid AUD and PTSD in human and animal studies is challenging. However, the development of advanced neuroimaging has enabled an assessment of structural and functional brain network architecture at an unprecedented level of detail. New theoretical frameworks combined with network approaches are needed to focus more on the dimensional and complex nature of brain disorders.[104]

Modeling the comorbid condition in nonhuman animals is crucial, because circuit manipulations and monitoring single-neuronal activity in specific pathways and cell types will provide a better snapshot of causal relationships between PTSD and AUD. Although several studies have used rodent models to examine comorbid PTSD and AUD,[105] preclinical studies have been challenging because of the wide array of stress procedures, different time courses of pathological behavior development, and individual differences within a model. However, technological progress in the next generation of optical, molecular, and observational tools offers a productive direction for future research using preclinical models. System-level interrogation with greater specificity may lead to identifying pathophysiological abnormalities and formulating coherent principles that explain the interactions between these disorders. Ultimately, the promise is that this knowledge may translate to hypothesis-driven, individual clinical interventions and therapeutic strategies for treating comorbid PTSD and AUD.

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Effects of Binge Drinking on the Developing Brain

Studies in Humans

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Binge drinking is a pattern of alcohol drinking that raises a person's blood alcohol concentration to at least .08%, which amounts to consuming five alcoholic drinks for men and four alcoholic drinks for women in about 2 hours. It is the most common form of alcohol misuse in adolescents and young adults. Heavy drinking includes the same criterion as binge drinking, but with higher frequency (i.e., 5 or more days in the past 30 days). Although binge drinking or heavy drinking alone is insufficient to meet the criteria for an alcohol use disorder (AUD) diagnosis, there are neurobiological changes, as well as an increased risk of developing an AUD later in life, associated with this form of alcohol misuse. This review describes the recent neuroimaging findings in binge drinking and heavy-drinking adolescents and young adults, a developmental period during which significant neuromaturation occurs.

Key words: Alcohol misuse; binge drinking; college drinking; neurodevelopment; neuroimaging; young adults

It has been well established that the brain undergoes significant maturation during adolescence that continues into young adulthood.[1] Studies using structural magnetic resonance imaging have described linear and nonlinear changes in cortical gray-matter volume and thickness[2-5] and increases in white-matter volume and integrity[2,6-9] occurring during development. Gray-matter volume peaks earlier in females (i.e., around age 11) than in males (i.e., around age 12) and declines during adolescence due to pruning of unused synaptic connections in order to promote efficient communication between neurons.[6] Furthermore, gray matter has been shown to reach earlier maturation in the sensorimotor cortices, whereas the frontal and temporal cortices mature later in development.[4] The prefrontal cortex, which is central to executive control, matures later compared with earlier developing limbic structures thought to be more involved in reward and emotional processing.[6,10,11] The asynchronous development of the prefrontal cortex and emotional and reward circuitry has been hypothesized to result in increased risk-taking behavior during adolescence, such as alcohol use.[12-15] This is especially of concern because ongoing neurodevelopment may render the adolescent brain particularly vulnerable to the neurotoxic effects of alcohol, as has been shown repeatedly in animal models.[16-19]

Binge drinking is a pattern of alcohol drinking that raises a person's blood alcohol concentration to at least .08%, which amounts to consuming five alcoholic drinks for men and four alcoholic drinks for women in about 2 hours.[20] It is the most common pattern of alcohol consumption in adolescents and young adults. As of 2014, 1.5 million adolescents ages 12 to 17 (6.1%) and 13.2 million young adults ages 18 to 25 (37.7%) in the United States reported binge drinking.[21] Heavy drinking includes the same criterion as binge drinking, but with higher frequency (i.e., 5 or more days in the past 30 days).[21] In the National Survey on Drug Use and Health, 257,000 adolescents (1%) and 3.8 million young adults (10.8%) reported heavy drinking.[21] Although binge or heavy drinking alone is insufficient to meet criteria for an alcohol use disorder (AUD) diagnosis, there are neurobiological changes, as well as an increased risk of developing an AUD later in life, associated with this form of alcohol misuse.[22] This article reviews neuroimaging studies assessing the effects of binge and heavy drinking on brain structure and function in adolescents. Studies in which participants met criteria for AUD were not included. Further, the age range included studies in adolescents and young adults, which extends up to a mean age of 25, because brain matu-

ration continues to occur well into the late 20s.[2]

Effects on Brain Structure—Gray Matter

Volume

Cross-sectional studies in binge drinking adolescents and college-age individuals have demonstrated regions of both more and less gray-matter volume compared with nondrinking peers, with volumes often related to frequency and quantity of alcohol consumption. For example, a recent study found that adolescents and young adults who consumed moderate to high levels of alcohol had smaller total-brain, frontal-lobe, and temporal-lobe volumes than their nondrinking peers; however, they also found that a greater number of lifetime drinks was positively associated with greater temporal-lobe volume.[9] In support of the notion that binge drinking is associated with lower gray-matter volume, a study of college-age binge drinkers found that higher Alcohol Use Disorders Identification Test (AUDIT) scores, indicative of greater reported frequency and quantity of alcohol consumption and alcohol-related problems, were associated with smaller frontal-lobe volumes.[23] An association between alcohol use and smaller gray-matter volume also was supported by another study that identified smaller precuneus volumes in a group of college-age binge drinkers compared with alcohol-naïve controls.[24] Further, greater AUDIT scores again were associated with smaller gray-matter volumes in the amygdala and hippocampus.[24] Additionally, among binge drinking adolescents, greater peak number of drinks in the past 3 months was associated with decreased cerebellar gray-matter volume.[25] Together, these findings suggest that binge drinking during development is associated with various regions of lower cortical, subcortical, and cerebellar brain volume, and that these changes often are associated with alcohol drinking characteristics.

Contrary to findings of smaller brain volumes, Howell and colleagues reported greater ventral striatal, thalamic, and lingual-gyrus volumes in college-age binge drinkers compared with control subjects.[24] A study on binge drinking, college-age participants also found increased frontal, occipital, anterior cingulate cortex (ACC), and posterior cingulate cortex volumes compared with nondrinking control subjects.[26] In this study, larger dorsolateral prefrontal cortex (DLPFC) volumes were positively associated with speed and quantity of alcohol consumption and negatively associated with age of onset of alcohol use.[26] It is worth noting that these individuals reported binge drinking for a minimum of 3 years prior to neuroimaging sessions, suggesting that volumetric increases in regional gray matter may be associated with long-term binge drinking.

In addition to these disparate findings in gray-matter volume, sex-specific effects also have been observed in college-age binge drinkers. Kvamme and colleagues noted a significant sex-by-drinking status interaction in numerous prefrontal, parietal, temporal, and striatal regions, such that binge drinking males had smaller volumes than alcohol-naïve males, whereas binge drinking females had larger volumes than alcohol-naïve females.[23] Although these sex-specific effects partially may explain the bidirectional effects seen in previous studies, there are likely many other factors that could contribute to these disparate findings, including the inability of cross-sectional designs to capture alterations in nonlinear developmental trajectories.[2-5]

To better address volume-related changes associated with drinking, longitudinal studies have begun to investigate gray-matter volume both before and after binge drinking. The first of such studies examined heavy-drinking adolescents with a baseline magnetic resonance imaging scan when the subjects were alcohol naïve and a follow-up scan approximately 3 years later, after binge drinking. At baseline, adolescents who later transitioned into heavy drinking had smaller ACC, posterior cingulate cortex, and inferior frontal gyrus (IFG) gray-matter volumes.[27] Furthermore, heavy-drinking adolescents showed accelerated reductions in the thalamus/hypothalamus, inferior temporal gyrus, middle temporal gyrus (miTG), caudate, and brain stem, with greater lifetime alcohol use associated with a greater reduction in gray-matter volume in the left caudate and brainstem.[27]

A follow-up to this study that investigated gray-matter volumes in heavy-drinking adolescents at baseline and during multiple follow-ups found that heavy drinkers exhibited greater reductions in overall neocortex volume, as well as in frontal, lateral frontal, and temporal cortex volumes.[28] Finally, Whelan and colleagues used machine-learning techniques to classify adolescents before and after initiation of binge drinking.[29] They reported that before alcohol use, binge drinking adolescents had lower gray-matter volume in the superior frontal gyri (SFG) and greater volume in the premotor cortex compared to nondrinking control subjects. After alcohol initiation, however, smaller ventral medial prefrontal cortex (PFC) and IFG volumes were observed compared with nondrinking controls.[29] Taken together, these findings suggest that binge drinking during development may result in accelerated decreases in gray-matter volume, above and beyond what is seen in typical maturation, likely caused by the neurotoxic effect of alcohol. It also is possible, based on evidence from cross-sectional studies in college-age individuals (described above), that a longer duration of alcohol use into young adulthood may result in greater gray-matter volumes in young adults who binge drink, potentially because of impaired synaptic pruning. Additional longitudinal studies with multiple time points will be necessary to elucidate alcohol's

effects on the full developmental trajectory across adolescence and young adulthood.

Cortical Thickness

Generally, studies investigating cortical thickness in binge drinking adolescents have supported findings of decreases in gray matter. Similar to their gray-matter volume findings noted above, Pfefferbaum and colleagues noted that alcohol-consuming adolescents had thinner total, frontal, temporal, and cingulate cortices than nondrinkers; moreover, the number of binge drinking episodes in the past year was negatively associated with frontal and parietal cortex thickness.[9] This finding is in agreement with another cross-sectional study of young adults, which determined that binge drinkers had thinner cortical measures in the ACC and posterior cingulate cortex compared with light drinkers (i.e., consuming one or two drinks per week, but no binge episodes).[30] Further, ACC cortical thickness was negatively correlated with the number of drinking occasions and number of drinks per occasion in the past 3 months, indicating that greater frequency and quantity of use is associated with thinner cortices.[30]

Similar to the volumetric study previously cited, sex-specific effects also have become apparent when investigating cortical thickness in binge drinking adolescents.[23] A cross-sectional study in binge drinkers identified sex-by-drinking status interactions for cortical-thickness measures in four frontal regions (i.e., frontal pole, pars orbitalis, medial orbital frontal, and rostral anterior cingulate). Thus, binge drinking males had thinner cortices than alcohol-naïve control subjects, whereas binge drinking females had thicker cortices than alcohol-naïve control subjects.[31] The directionality of these findings is consistent with those of Kvamme and colleagues.[23] The findings suggest that during this particular window of development, alcohol may have differential effects for boys and girls, likely resulting from underlying sex differences in the rate and timing of synaptic pruning in adolescents.[6]

In a longitudinal investigation of the effects of binge drinking on cortical thickness, Luciana and colleagues found that adolescents who initiated alcohol use showed a significantly greater decrease in middle frontal gyrus (miFG) cortical thickness between baseline and revisit compared with adolescents who remained alcohol naïve,[32] suggesting that alcohol has a neurotoxic effect on frontal lobe development. However, this study found no differences in cortical thickness prior to initiation of alcohol use, contrary to a subsequent study observing differences in baseline gray-matter volume.[27] Other studies have investigated the effects of binge drinking on cortical thickness in a longitudinal manner, but without an alcohol-naïve baseline. Jacobus and colleagues examined cortical thickness over 3 years and found that concomitantly binge drinking and marijuana using adolescents had thicker cortices across time in five frontal, eight parietal, one temporal, and one occipital region compared with alcohol- and marijuana-naïve control subjects.[33] Moreover, in three frontal regions, control subjects showed a decrease in cortical thickness across time, whereas concomitantly binge drinking and marijuana using adolescents did not. A prior study had suggested that these effects persisted following abstinence, because concomitantly binge drinking and marijuana using adolescents showed greater thickness in the ACC, medial temporal gyrus, lingual gyrus, and occipital cortex both before and after 28 days of monitored abstinence.[34]

Taken together, these studies suggest that, when combined with marijuana use, binge drinking may result in increases, as opposed to decreases, in cortical thickness, that these increases are cumulative with prolonged use, and that they persist even following a month of abstinence. Furthermore, although these studies contradict some literature,[9,30,32] they may help provide an alternative explanation for the equivocal findings in gray-matter volume described above. In fact, in the longitudinal study by Squeglia and colleagues, although a greater number of lifetime alcohol-use occasions was associated with greater reductions in caudate and brainstem volume, a greater number of lifetime marijuana uses was associated with increases in caudate volume.[27] This provides further evidence that although gray-matter volume and thickness typically decrease in binge drinking adolescents and young adults, concomitant marijuana use may result in observed increased volume and thickness.

Effects on Brain Structure—White Matter

Volume

As opposed to the varied findings in gray-matter volume, results in white-matter volume have been more parsimonious. Cross-sectional studies have shown that a greater number of lifetime drinks was associated with smaller central white-matter volume,[9] and peak number of drinks during a binge episode in the past 3 months was associated with smaller cerebellar volumes.[25] Longitudinal studies tell a similar story, with binge drinking adolescents showing reduced white-matter volumes both before[27] and following initiation of binge drinking.[28,32] Squeglia and colleagues found that heavy-drinking adolescents had lower baseline cerebellar white-matter volumes compared with control subjects, but the investigators identified no regions where white-matter volume changed differentially across time.[27] However, in a follow-up study, heavy-drinking adolescents exhibited significantly attenuated white-matter growth in the pons and corpus callosum between baseline and follow-up scans, compared with controls.[28] Luciana and colleagues reported similar findings, such that alcohol-naïve controls showed an increase in vol-

ume in white-matter regions of the precentral gyrus, miTG, SFG, and lingual gyrus between baseline and follow-up, whereas binge drinking adolescents did not.[32] Taken together, these observations suggest that reduced white-matter volume may precede alcohol use, and that alcohol use during adolescence attenuates the typical maturational increase in white-matter volume observed in adolescence in a dose-related fashion.[2,6-8]

Microstructure

Varied differences in white-matter microstructure have been observed between binge drinking adolescents (with and without concomitant marijuana use) and non–alcohol using controls. First, a cross-sectional diffusion tensor imaging study investigating fractional anisotropy (FA)—a measure thought to reflect white-matter myelination and axonal integrity and coherence—found that binge drinking adolescents had lower FA than control subjects in seven frontal, three parietal, two temporal, four subcortical, and two cerebellar regions. Furthermore, in six of these regions, lower FA was associated with significantly greater lifetime hangover symptoms and higher estimated peak blood alcohol concentrations.[35]

In a second cross-sectional study, concomitant binge drinking and substance using adolescents had lower FA than control subjects in 10 separate frontal, parietal, temporal, and subcortical regions, and reduced FA in these regions was associated with greater lifetime alcohol use.[36] Interestingly, the investigators also noted three regions (i.e., the superior longitudinal fasciculus, internal capsule, and occipital lobe) where FA was greater in concomitant binge drinking and substance using adolescents than in control subjects, and they found that greater FA in these regions was associated with greater lifetime alcohol use.

Finally, a third cross-sectional study of binge drinking adolescents and concomitant binge drinking and substance using adolescents found that binge drinking adolescents, again, had lower FA than control subjects in eight different regions, including the superior corona radiata (SCR), inferior longitudinal fasciculus, superior longitudinal fasciculus (SLF), inferior fronto-occipital fasciculus (IFOF), and cerebellar peduncle.[37] Those with concomitant substance use, in contrast, only had significantly lower FA (compared with control subjects) in three regions, including the SCR and SLF, and they had significantly higher FA than binge drinking adolescents in four regions (i.e., the SCR, SLF, IFOF, and cerebellar peduncle). In this study, greater marijuana use frequency was associated with greater FA in the SCR and SLF, whereas a greater number of lifetime drinks was associated with greater FA in the SLF. Together, these findings suggest that binge drinking during adolescence is associated with reduced FA, but that concomitant marijuana use may interact with the effects of alcohol, resulting in an alteration of this effect.

These cross-sectional findings have been corroborated by numerous longitudinal studies. Luciana and colleagues reported that compared with control subjects, adolescent binge drinkers showed significantly diminished normative increases in FA in the dorsal caudate and IFOF between baseline and follow-up visit.[32] Another study found that concomitant binge drinking and substance using adolescents had reduced FA in the corpus callosum, prefrontal thalamic fibers, and posterior corona radiata at follow-up, compared with control subjects, with no differences reported at baseline.[38]

A series of studies examined FA in a group of binge drinking and concomitant binge drinking and substance using adolescents and young adults at baseline and follow-up.[39-41] First, they found that binge drinking adolescents both with and without concomitant substance use showed a significant, widespread decline in FA across the three visits, resulting in lower FA after 3 years of use compared with control subjects.[39] Moreover, lower FA in the fornix and SCR at baseline in concomitant binge drinking and substance using adolescents predicted greater subsequent use at the first follow-up, above and beyond baseline substance use.[40] It is important to note that in these two studies,[39] adolescent binge drinkers and substance users were not drug and alcohol naïve at baseline; rather, they were drinking and using marijuana throughout the entirety of the study. Lastly, Jacobus and colleagues identified 20 regions in the brain where there was a significant group-by-time interaction, such that adolescents who used both alcohol and marijuana concomitantly showed a sharper decline in FA between baseline and 3-year follow-up than those who only binge drank.[41] In combination, these findings suggest that whereas binge drinking during adolescence and young adulthood appears to be associated with reduced FA, results tend to be less clear when adolescents concomitantly use marijuana. Whereas Jacobus and colleagues found that binge drinkers with concomitant marijuana use initially had had greater FA than those who only binge drank,[37] a longer history of concomitant marijuana use, extending into young adulthood, may eventually result in a steeper decline in FA across development.[41]

Effects on Brain Function

Verbal Encoding

Learning and memory abilities are crucial for an adolescent's success, and development of those abilities may be altered or attenuated by alcohol use. Verbal encoding/learning, using a verbal paired-association task, has been used to investigate the impact of alcohol on learning and memory in binge drinking adolescents with and without comorbid marijuana use. A preliminary study found that binge drinking adolescents had greater activation in the SFG, superior parietal lobule,

inferior parietal lobule (IPL), and the cingulate, as well as lower activation in one cluster encompassing the cuneus, precuneus, lingual gyrus, and parahippocampal gyrus (PHG) during novel word encoding.[42]

In a follow-up investigation, Schweinsburg and colleagues found that binge drinking and concomitant binge drinking and substance using adolescents, when compared with marijuana-only users and control subjects, showed greater encoding-related activation in the postcentral gyrus, IPL, and SFG, and less activation in the fusiform gyrus, PHG, cuneus, precuneus, IPL, IFG, precentral gyrus, and cingulate.[43] They also identified regions of the brain (i.e., the IFG, miFG, SFG, and cuneus) where users of either alcohol or marijuana showed greater brain response than nonusers during novel word encoding, whereas users of both substances resembled nonusers. Because performance on the task was the same between binge drinkers and control subjects,[42,43] these findings suggest that alcohol use during adolescence may cause adolescents to adopt a different neural strategy (e.g., heavier prefrontal-cortex recruitment) to achieve the same successful verbal encoding. Because of the cross-sectional design, it is unknown whether these differences were present prior to or developed as a consequence of alcohol consumption.

Working Memory

Brain response during working memory also has been shown to be altered in binge drinking adolescents and young adults. In a preliminary study, Tapert and colleagues found that brain response during a visual working memory task was negatively associated with subjective response to alcohol, such that adolescents who reported that a greater quantity of alcohol was needed to feel an effect showed greater activation in the SFG, cingulate, cerebellum, and PHG during memory retrieval.[44] A subsequent study showed that binge drinking adolescents had greater activation in the medial frontal gyrus (meFG), SFG, IPL, and supramarginal gyrus, as well as less activation in the middle occipital gyrus, when compared with control subjects.[45] Furthermore, in longitudinal analyses, binge drinking adolescents actually had lower activation in the IPL and meFG at baseline (i.e., prior to drinking), but when compared with control subjects, they showed a greater increase across time. These greater increases in brain activation were associated with a greater peak number of drinks in the past year, more past-month drinking days, and greater withdrawal/hangover symptoms at follow-up.[45] Further, less premorbid activation in the meFG and IPL predicted a higher peak number of drinks and drinking days in the year preceding follow-up.[45] This suggests that binge drinking not only affects neural response during working memory, but that baseline differences in brain activation during working memory may be useful in identifying adolescents who may go on to drink.

These findings also are supported by cross-sectional work using other working memory tasks. One study found that during verbal working memory, binge drinking young adults had greater activation in the parietal cortex (pre–supplementary motor area) than control subjects.[46] Moreover, more drinks per drinking occasion were associated with greater dorsal medial PFC activation, whereas more drinking occasions per week were associated with greater cerebellar, thalamic, and insular activation. In contrast, Squeglia and colleagues reported that binge drinking adolescents had lower activation in the SFG and IFG compared with control subjects.[47] However, this study differed in two ways from the previous studies. Squeglia and colleagues used a spatial working memory task and also reported significant sex differences, such that binge drinking females showed less activation than control subjects, and binge drinking males showed greater activation than control subjects in the SFG, IFG, ACC, miFG, miTG, superior temporal gyrus, and cerebellum. These findings suggest that, in general, adolescents show alcohol-related increases in activation, particularly in fronto-parietal networks during working memory; however, at least for spatial working memory, these findings may be sex specific. Further work is necessary to tease out the different elements (e.g., spatial versus verbal) of working memory and the effects of alcohol on their associated neural responses.

Risk Taking and Reward Response

Because adolescence is a time of increased risk taking, including experimentation with alcohol, it may come as no surprise that binge drinking adolescents show altered brain response during various phases of risk taking. Whereas some investigators have attempted to elucidate binge drinking's effects on a particular aspect of risk-taking behavior,[48-50] others have investigated risk taking more broadly.[51] In a study looking at risk-taking behavior using the Iowa Gambling Task, binge drinking adolescents had greater risk-related activation in the amygdala and insula compared with control subjects, and they had more reported drinking problems related to less activation in the orbitofrontal cortex (OFC) and more activation in the insula.[51] Two recent studies separately investigated the effects of binge drinking during adolescence during decision making and reward receipt. In the first study, binge drinking adolescents, compared with control subjects, showed reduced cerebellar response during reward receipt following initiation of binge drinking, a finding that remained significant when controlling for premorbid activation, and which was associated with more drinks per drinking day in the past 90 days.[48]

A longitudinal investigation found that binge drinking adolescents, compared with control subjects, had lower

activation in the IFG, IPL, miTG, and superior temporal gyrus across time, suggesting a different pattern of brain activation that occurs prior to binge drinking and persists after alcohol initiation.[49] There also was a significant group-by-time interaction in the dorsal caudate, such that binge drinking adolescents showed similar risky decision-making–related brain responses as controls at baseline, but they showed a reduced response following binge drinking. This reduction was associated with a greater number of drinking days and heavy drinking days in the previous 3 months.

Further, Worbe and colleagues used a novel risk-taking gambling task in binge drinking young adults to investigate brain responses during the decision-making and feedback phases of both reward and loss gambles.[50] During decision making in conditions with both a low and high potential for a loss, the study found that binge drinkers had greater activation in the OFC, superior parietal cortex, and DLPFC compared with control subjects. This finding was accompanied by more risky decisions during high-loss selections. Furthermore, although giving feedback during the task reduced the amount of risky decisions in binge drinking young adults, it also was associated with greater activity in the IFG and IPL, when compared with control subjects.

In addition to studies looking at adolescent risk-taking behavior, a study by Whelan and colleagues investigated brain responses during reward anticipation and receipt outside of the context of risk, using the monetary incentive delay task.[29] The study demonstrated that, compared with control subjects, adolescent binge drinkers had greater activation during reward receipt in the SFG prior to initiation of binge drinking, but they had reduced activation during reward anticipation and receipt in the ventral medial PFC and IFG after binge drinking. Taken together, these findings suggest that binge drinking during adolescence and young adulthood is associated with alcohol-related alterations in brain response during decision making and reward/consequence notification. Further, group differences in fronto-parietal brain response during risky decision making and reward receipt that occur prior to drinking may serve as a risk factor for future drinking.[29,49]

Inhibition

Several longitudinal studies have used a standard go/no-go procedure to investigate the effects of binge drinking on brain response during inhibition. One study found that, at baseline, adolescents who went on to engage in heavy drinking had reduced brain response during successful inhibition in the DLPFC, miFG, SFG, IFG, meFG, paracentral lobules, cingulate, putamen, miTG, IPL, and pons, compared with adolescents who remained alcohol naïve.[52] In another study, less activation during successful inhibition in the ventral medial PFC predicted more alcohol dependence symptoms in heavy-drinking adolescents at 18-month follow-up.[53] Meanwhile, in a study investigating the failure to inhibit responding, greater activation in the premotor cortex served as a risk factor for adolescents who later went on to engage in binge drinking.[29] Together, these studies suggest that lower engagement of numerous regions, particularly within the fronto-parietal network, during successful inhibition, as well as greater engagement of premotor regions during unsuccessful inhibition, may precede the onset of binge drinking.

Furthermore, compared with alcohol-naïve control subjects, heavy-drinking adolescents were shown to have significantly lower levels of brain activation during inhibition in the miFG, IPL, putamen, and cerebellum at baseline.[54] They also showed greater increases in inhibition-related brain responses, compared to controls, following initiation of heavy drinking. Greater increases in brain response during response inhibition between baseline and follow-up were associated with more lifetime drinks. The same group of researchers also found that these patterns of activation differed in adolescents who experienced alcohol-induced blackouts. Prior to initiation of heavy drinking, adolescents who did and did not experience alcohol-induced blackouts showed less activation in the IPL compared with control subjects.[55] However, adolescents who went on to experience alcohol-induced blackouts showed greater activation during inhibition in the miFG, miTG, cerebellum, and parietal cortex (pre–supplementary motor area) compared with those who did not experience blackouts. These findings suggest that adolescents who later experience alcohol-induced blackouts show patterns of brain activation during inhibition, which may render them more vulnerable to the memory-impairing effects of alcohol.

Lastly, a recent study in binge drinking young adults found that those who escalated drinking over a 12-month period had greater fronto-parietal activation during inhibition compared with young adults who maintained stable drinking levels.[56] Taken together, it appears that hypoactivation of the fronto-parietal network during inhibition may serve as a risk factor for alcohol use initiation; however, after alcohol use initiation, hyperactivation of the fronto-parietal network during inhibition may serve as a risk factor for escalation of drinking.

Cue Reactivity

Two recent studies have looked at brain activation elicited by an alcohol cue (i.e., cue reactivity), using an alcohol pictures task, in binge drinking adolescents and young adults. Dager and colleagues found that young adults who transitioned from moderate to heavy drinking over a 1-year follow-up had greater activation at baseline in the caudate, ACC, medial prefrontal cortex, precentral gyrus, insula, IFG, and OFC, compared with those who

remained moderate drinkers or heavy drinkers throughout the study.[57] Furthermore, brain activation in this network of regions predicted future drinking and alcohol-related problems, above and beyond baseline drinking characteristics. This suggests that changes in how the brain responds to alcohol cues may help predict which individuals may transition from light to heavy drinking and may be more informative than simply comparing heavy drinkers with control subjects. In another study, heavy-drinking adolescents had greater cue-elicited brain response in the dorsal striatum, cerebellum, PHG, and thalamus than control subjects prior to abstinence; however, the group differences in the cerebellum and ACC no longer remained significant after 28 days of abstinence.[58] This suggests that although cue-elicited brain response may be a predictor of future drinking, if adolescents manage to maintain abstinence, they may be able to reduce that cue-elicited response. This finding has important implications for future intervention strategies.

Effects on Behavior and Cognition

Many of the structural and functional differences observed in adolescent binge drinkers also are associated with changes in cognition and behavior. Several studies have examined neurocognitive changes related to binge drinking and reported poorer performance in many domains, including attention,[59,60] learning and memory,[59,61-66] and visuospatial functioning.[60] Neuroimaging studies have found that the poorer sustained attention observed in binge drinking adolescents is associated with thicker PFCs[31] and lower FA in the inferior longitudinal fasciculus[67]—regions where thickness and FA differed significantly between binge drinking adolescents and control subjects. This suggests that binge drinking during adolescence may cause a delay in the maturation of both gray and white matter, resulting in poorer sustained attention.

Furthermore, binge drinking adolescents and young adults have demonstrated impaired performance on a variety of learning and memory tasks.[59,61,62,64,65] These findings also have been associated with changes in brain structure in binge drinking adolescents in regions of the brain where these adolescents differ from control subjects. Binge drinking–related deficits in working memory also have been demonstrated,[61,63] with one study showing that after 3 years of binge drinking, greater gray-matter volume in the DLPFC was positively associated with working-memory errors.[26] Further, decreased FA in the inferior longitudinal fasciculus in binge drinking and substance using adolescents has been shown to be associated with poorer working-memory performance.[67] In addition, although an initial study found that the number of drinking days in the past year predicted greater reductions in performance on a visuospatial task,[60] a follow-up study showed that thicker frontal cortices corresponded with poorer visuospatial performance in binge drinking females.[31] These findings suggest that delayed cortical maturation may underlie the effects of binge drinking on visuospatial performance.

Binge drinking adolescents also demonstrate impaired, or riskier, decision making,[68] likely resulting from impairments in impulsivity[69] and inhibition.[64] One study found that young adults who showed stable, high levels of binge drinking made riskier choices on the Iowa Gambling Task compared with adolescents who engaged in stable, low levels of binge drinking.[68] Other studies have reported that heavy-drinking adolescents show greater impulsivity than light drinkers[69] and that binge drinking adolescents show impaired inhibition compared with control subjects.[64]

Neuroimaging studies have helped shed some light on the mechanisms underlying this impaired decision making and impulse control. Structurally, greater impulsivity in adolescent binge drinkers has been shown to be associated with smaller DLPFC and IPL volumes and greater dorsal cingulate and precuneus volumes,[70] whereas reduced FA in the fornix of concomitant binge drinking and substance using adolescents has been shown to predict greater amounts of risky behavior a year and a half later.[40] Functionally, riskier behavior on the Iowa Gambling Task in binge drinking adolescents has been accompanied by greater activation in the insula and amygdala, when compared with control subjects.[51] Also, as described above, greater activation in the OFC, superior parietal cortex, and DLPFC, when compared with controls, has been associated with more risky decisions when there was a high potential for loss.[50] Taken together, these findings suggest that the underdevelopment of control regions (e.g., smaller DLPFC and IPL volumes) and hyperactivation of reward-salience regions (e.g., amygdala), both of which are hallmarks of adolescent neurodevelopment, may be exacerbated in adolescents who binge drink and may underlie the observed increase in risk-taking behavior in binge drinking adolescents.

Conclusions

Although evidence is still emerging on how binge drinking during adolescence and young adulthood affects the brain, many general conclusions can be drawn from current literature (for a summary of all replicated findings in binge drinking adolescents and young adults, see Figure 1). First, binge drinking during adolescence appears to result in a decrease in both gray-matter volume and cortical gray-matter thickness,[9,30] with longitudinal studies suggesting that some of these differences may be present prior to binge drinking and continue to worsen as adolescents initiate alcohol consumption.[27,28,32] Although it must be noted that some studies show increased gray-matter volume or thickness in binge drinking

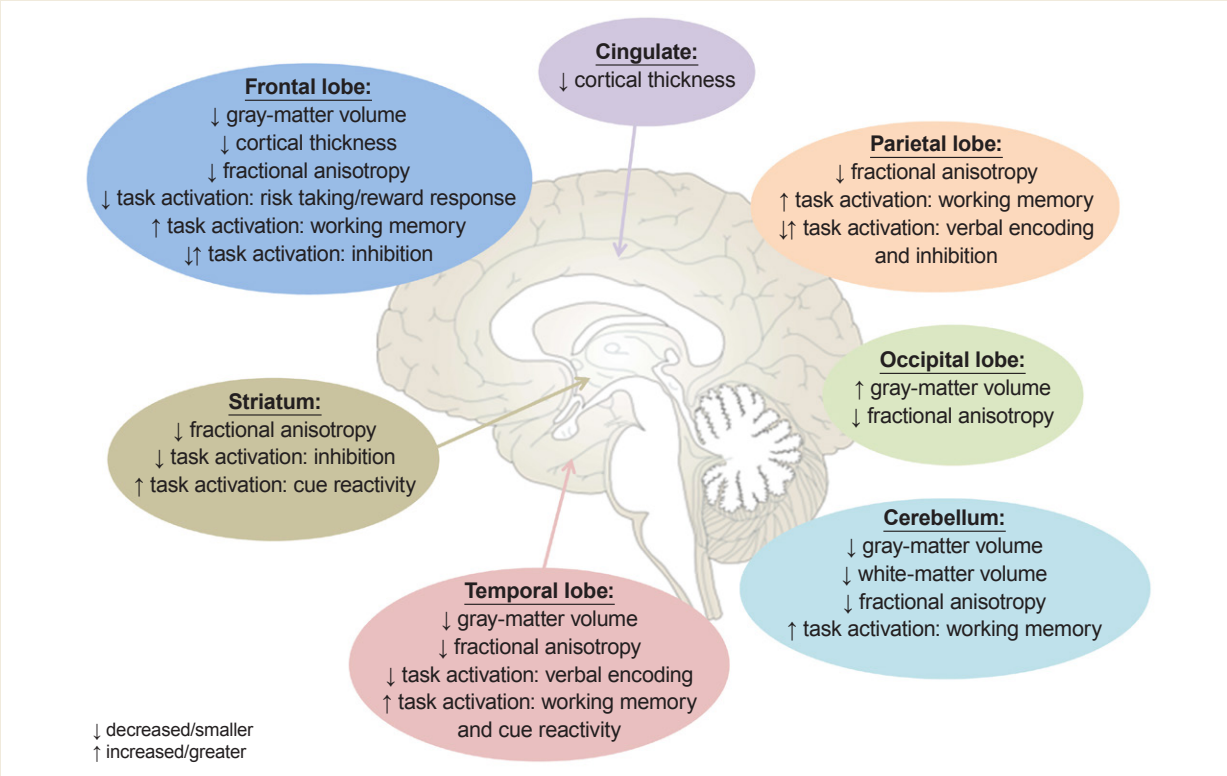

Figure 1 Replicated findings in binge drinking adolescents and young adults.

adolescents, it is plausible that these contradictory findings either are caused by the influence of concomitant marijuana use[33,34] or are the result of examining the effects of binge drinking on a nonlinear developmental pattern[2-5] in a cross-sectional manner.[24,26]

Second, multiple studies consistently have shown that the developmental increases in white-matter volume, often observed in adolescents,[2,6-8] appear to be attenuated in adolescents who binge drink,[27,28,32] and that this attenuation is associated with the degree of substance use.[9,25] However, studies demonstrating altered white-matter microstructure in binge drinking adolescents have yielded mixed results, showing both increases and decreases in FA. Again, it appears that this may partially be explained by the presence of concomitant marijuana use in adolescence.[36,38-41] More studies comparing concomitant users to those using only alcohol or marijuana likely are necessary to completely disentangle these effects.

Functionally, binge drinking during adolescence appears to affect brain responses in numerous regions, across a variety of tasks. Cross-sectional work has identified both increased and decreased brain activation in multiple task domains (e.g., verbal learning, working memory, risk taking, cue reactivity, and inhibition) and demonstrates the necessity of longitudinal studies to determine which effects are a result of alcohol consumption and which reflect an underlying risk phenotype for those who will go on to binge drink. Longitudinal work, specifically in working memory[45] and response inhibition,[52,54] suggests that binge drinking adolescents demonstrate similar or lower levels of brain activation in task-relevant regions at baseline, followed by an exacerbated increase in activation, above and beyond that seen in control subjects, after initiation of binge drinking. A failure to recruit task-relevant regions at baseline in future binge drinkers could lead to poorer task performance, while hyperactivation following alcohol use suggests that binge drinking adolescents require more recruitment of task-relevant networks to achieve desired cognitive outcomes.

Meanwhile, similar or lower levels of brain activation during risk-taking behavior (i.e., risky decision making and reward response) also have been observed in binge drinking adolescents.[48,49] However, unlike during working memory and response inhibition, binge drinking adolescents have lower levels of brain response over time during risky decision making and

reward response. This may suggest not only a pattern of activation during risky decision making that may serve as a risk factor for future drinking,[49] but also a diminished brain response to risky stimuli and rewards following binge drinking.[48,49] This decreased brain response may be what causes binge drinking adolescents to show greater risky behavior and may enhance reward seeking.

Understanding these altered neurobiological features in binge drinking adolescents is extremely relevant, because changes in both brain structure and function have been related to changes in cognition in binge drinking adolescents.[26,31,40,50,51,60,67,70] Moreover, not only do differences in task activation serve as risk factors for future drinking,[45,49,52,54] but neurobiological features, such as fronto-parietal hyperactivation during inhibition and atypical white-matter microstructure, may serve as risk factors for escalated drinking and risk-taking behavior in adolescents who are already drinking.[40,56] Adolescent onset of alcohol use has been associated with an increased risk for developing an AUD later in life;[22] thus, understanding neurobiological markers that are associated with both initiation and escalation of alcohol use is important for advancing future prevention and intervention strategies in an effort to reduce the rates of AUD.

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Binge Drinking's Effects on the Developing Brain—Animal Models

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Adolescence typically is a time of experimentation, including alcohol use and, particularly, binge drinking. Because the brain is still developing during adolescence, such exposure could have long-lasting effects. Animal models and adolescent intermittent ethanol exposure (AIE) paradigms have been used to help elucidate the consequences of adolescent binge drinking. These studies have identified cognitive deficits, particularly in challenging cognitive tasks, and behavioral alterations such as greater risk preferences, impulsivity, and disinhibition. AIE also is associated with changes in affect when the animals reach adulthood, including increased social anxiety and, sometimes, general anxiety. Animal models have demonstrated that AIE can result in retention of certain alcohol-related adolescent phenotypes (i.e., reduced sensitivity to alcohol's aversive effects and increased sensitivity to alcohol's rewarding effects) into adulthood, which may motivate continued elevated alcohol use. The detrimental effects of adolescent alcohol exposure extend to a diversity of lasting alterations in the brain, including reduced neurogenesis, increased proinflammatory responses, changes in gene expression through epigenetic mechanisms, and alterations in the activities of various neurotransmitter systems. Further exploration of these mechanisms in animal models and humans may lead to improved prevention and intervention efforts.

Key words: Adolescence; alcohol exposure; alcohol use disorder; animal studies; binge drinking; brain development

Adolescence typically is a time of experimentation and emulation of adult behaviors, and many adolescents initiate alcohol and other drug (AOD) use during this developmental period. Brain development continues during adolescence, which could render the adolescent brain particularly vulnerable to alcohol's effects. Consequently, adolescent alcohol exposure could result in long-lasting changes in neuropsychological function and increased risk of developing alcohol use disorder (AUD). To better understand and minimize these risks, it is crucial to comprehensively study alcohol's impact on the adolescent brain. Such studies in humans face a number of challenges, however. For example, ethical constraints prevent the administration of alcohol to underage youth. Moreover, in human adolescents it is difficult to discern whether observed correlations between alcohol use and the behavioral or neuropsychological measures under investigation reflect causes or consequences of alcohol use or are purely coincidental. Finally, despite significant progress in noninvasive imaging technologies, the complexity of the human brain and technical limitations of brain analyses hamper researchers' abilities to fully investigate how alcohol influences adolescent brain structure and function.

Animal models using laboratory animals such as mice and rats can help circumvent some of these problems. However, their use also is associated with certain limitations. Most importantly, no currently available animal model can fully represent complex human behaviors such as alcohol use and addiction. Certain factors that influence adolescent neurobehavioral function and AOD misuse are not amenable to analysis using animal models, including variables such as verbal ability and language, and influences such as self-esteem, culture, media, or even parenting styles. Despite these limitations, much of what is currently known about the intricacies of brain development, neural substrates of AOD use and misuse, and adolescent responses to AODs has been obtained using animal models. This article summarizes some of the characteristics of animal models for studying alcohol's effects on the adolescent brain and reviews the findings of studies using those models that have shed light on functional and structural alterations

associated with adolescent alcohol use, the alcohol-induced persistence of adolescent phenotypes into adulthood, and the impact of adolescent alcohol use on later alcohol consumption.

Characteristics of Animal Models

The potential usefulness and validity of animal models, especially for complex behaviors such as alcohol misuse and its consequences, depend primarily on the specific research questions being asked. The validity of such models can be assessed on three levels:[1]

- Face validity assesses whether the phenomenon under study in the model resembles the targeted human behavior in terms of its behavioral, cognitive, and physiological features. However, it is important to realize that even if certain behaviors or other effects appear similar across species, they may not share the same underlying mechanisms.
- The measure of construct validity focuses on the relevance of the phenomenon under investigation in the animal model to the concept being modeled. Investigators seek to determine how similar the animal model is to the biological foundation and neural underpinnings of the human behavior being modeled. This concept also considers the impact of moderators, such as previous experiences or the environment.
- The concept of predictive validity reflects how effectively the animal model predicts experimental findings or treatment outcomes in humans.

Assessment of the validity of animal models of adolescent alcohol consumption and its consequences is an ongoing, iterative process, as research in these areas escalates in both human adolescents and laboratory animals. The current research supports cautious optimism in the use of such models. For example, findings have shown signs of consilience between human adolescents and rodent models of adolescence when comparable assessment measures of alcohol sensitivity and consequences were used.[2]

Animal Models of Alcohol Use and Its Consequences

One of the main factors for using rodent animal models for alcohol research is that these animals voluntarily self-administer AODs when given free access. For example, rodents often orally self-administer substantial amounts of alcohol, particularly if they are offered beer or sweetened beverages. Laboratory animals and humans exhibit similar behavioral and cognitive responses to acute AOD administration. Laboratory animals effectively model a broad diversity of alcohol effects seen in humans, ranging from euphoria and social stimulation at low alcohol levels to intoxication, motor impairment, sedation, and memory impairment at higher doses.[3] In addition, animals that are chronically exposed to AODs can develop physical dependence, characterized by dysphoria and physical signs of withdrawal (e.g., tremor, anxiety, insomnia, and even seizures) when access is terminated.[4] Such physical dependence can be accompanied by a tendency for relapse, particularly after re-exposure to the drug or exposure to stressors or drug-related cues. Experiments that used a conditioned place preference approach demonstrated that laboratory animals, even without physical dependence, can develop a preference for contextual cues associated with drug use.

Not only are the behavioral consequences of alcohol exposure often similar in humans and in animal models, but the neural substrates underlying these effects also exhibit across-species similarities. Numerous studies have identified sufficient similarities in brain structure and function between rodents and humans to support the validity of animal models in assessing the consequences of alcohol use on the brain. For instance, consider the prefrontal cortex (PFC), a brain region that comprises a notably greater proportion of the total brain matter in humans and other primates than in rodents. In humans, the PFC is thought to play a central role in executive functions, such as working memory, temporal processing, planning, flexibility, and decision-making, which influence behaviors such as drug self-administration and dependence. Comparative studies have indicated that rats also engage in these behaviors, and that the PFC is critical for mediating these processes in rodents, nonhuman primates, and humans.[5,6] In rats and humans, the PFC can be divided into subregions that are associated with similar cognitive functions across species.[5] Experimental animal models have been used successfully to reproduce features of neuropathological and neurochemical changes observed in humans who had neurodegenerative and psychiatric disorders that affected their cognitive function.[7]

Extensive studies also have established the relevance of animal models for investigating drug use behaviors and the consequences. For instance, brain reward systems using the neurotransmitter dopamine, including dopamine projection regions of the nucleus accumbens (NAc), amygdala, and PFC, are critically involved in drug self-administration and dependence in humans and animal models.[8-10] In addition, in humans and laboratory animals, specific brain structures and neurochemical systems are critical for different aspects of alcohol use and misuse (e.g., producing dependence or mediating craving and relapse).[11]

However, differences exist between the rodent and the human and nonhuman primate brains that should be considered when translating findings from animal studies to the neurological substrates and consequences of alcohol use in humans. For example, electrophysiological studies have suggested that the medial PFC in the rat brain combines elements (i.e., the anterior cingulate cortex and the dorsolateral PFC) that are separated in the primate brain.[12]

Animal Models of Adolescence

Adolescence—that is, the transition from dependence on parents to the independence of adulthood—is not unique to humans and is, to some extent, experienced by all mammals. Similar biological changes, including alterations in the brain, are seen across a variety of mammalian species during adolescence.[13-15] Adolescence-associated neural alterations include regionally specific reductions in the number of synaptic connections between neurons and declines in the relative volume of certain cortical and subcortical areas.[14] Speed of information flow across distant brain regions increases,[14] as does the reactivity of some subcortical brain regions, including the NAc and amygdala.[13,15]

Adolescence-associated changes in dopamine-terminal regions, such as the amygdala and NAc, are particularly important in the context of adolescent AOD use, because these regions are critically involved in processing and responding to rewarding, aversive, and emotionally arousing stimuli, including social stimuli. In adolescents, when compared with adults, these brain regions often react in an exaggerated way to motivational stimuli.[13,15] In contrast, maturation of cognitive control regions in the PFC and other frontal regions occurs gradually during adolescence.[16] This maturational dissociation is thought to contribute to adolescent-characteristic behaviors, such as increased risk-taking and exploratory drug use.[17]

Such developmental alterations have been observed in humans and in animal models and have been matched by analogous behavioral changes in various species. Adolescent rats, for instance, show more peer-directed interactions, novelty-seeking or risk-taking behaviors, and consummatory behavior; find social stimuli, novel stimuli, and pleasant tastes particularly reinforcing; and voluntarily consume two to three times more alcohol than adult rats.[18-21]

Despite such similarities, there are, of course, marked differences between humans and rodents in the duration of this developmental period. Adolescence is relatively brief in rodents and in other mammals with a short life span. Adolescence in rats has been estimated to last only about a month (i.e., postnatal day [P] 25 to P55), with early to mid-adolescence ending at about P42, and late adolescence occurring from P43 to P55.[22] The experimental designs used to study adolescent alcohol use and its consequences, such as analyses involving operant self-administration, must be adapted to this relatively short time period.

To ensure the face validity of models, experimental designs for modeling human alcohol use and its consequences in animals must consider human drinking patterns. For example, alcohol misuse among human adolescents typically takes the form of binge drinking on weekends rather than daily drinking. This human adolescent behavior can be modeled by intermittent alcohol exposure. However, alcohol misuse among adults often involves more regular drinking patterns, which may be better represented by more continuous exposure models.

Despite these constraints, judicious use of animal models can complement studies in human adolescents and address questions that are ethically or technically not amenable to study in humans. Studies using animal models have identified numerous functional alterations associated with adolescent alcohol use, as well as a variety of neural alterations.

Functional Alterations Associated With Adolescent Alcohol Exposure

Studies of the lasting consequences of repeated alcohol exposure during adolescence in animal models have identified numerous functional alterations across domains, ranging from cognition and behavior, to affect, and to later alcohol consumption. These studies typically use alcohol exposure levels that produce blood ethanol concentrations of .08% or more—the level required to meet the definition for binge drinking specified by the National Institute on Alcohol Abuse and Alcoholism[23] (see **Drinking Patterns and Their Definitions** in this issue). Blood ethanol concentrations in these studies often average .15% to .20%, which is well within the binge-drinking range observed in field studies of human adolescents.[24] Usually, each alcohol exposure during a rat's adolescence is followed by a short period of abstinence before the next exposure period, a design sometimes called adolescent intermittent ethanol exposure (AIE).

Cognitive and Behavioral Alterations

Animal studies have helped identify a variety of cognitive deficits resulting from repeated adolescent alcohol exposure, particularly deficits in tasks that are thought to require hippocampal functioning.[25] Other identified deficits reflect aspects of executive functioning, where prefrontal cortical brain regions are thought to play a particularly important role.[16] Interestingly, the observed effects are highly specific. Learning of some less cognitively challenging tasks, such as passive avoidance or simple operant conditioning tasks, does not seem to be affected by adolescent alcohol exposure.[26,27] Alcohol-exposed animals sometimes exhibit deficits on more challenging tasks, such as conditional discrimination and object recognition tasks.[28] For adolescent animals exposed to ethanol, tasks that demand some degree of cognitive flexibility or self-control seem to be particularly vulnerable to performance impairment. These tasks include reversal learning,[29] extinction, and set-shifting tasks.[30] Adolescent alcohol exposure also is associated with a greater vulnerability to disruptions in spatial memory that are induced by ethanol challenge in adulthood.[25]

Other studies have assessed the effects of AIE on risk-taking behavior, impulsivity, and disinhibition, all behavioral propensities that could promote experimentation with AODs. Such studies have demonstrated that animals with adolescent alcohol exposure exhibited greater risk preferences on a probability-discounting task.[31,32] AIE has been associated with increased impulsivity and greater disinhibition, as indicated by an increase in time spent in open or lighted test areas.[30,32-34]

Changes in Affect

Animal studies also have demonstrated changes in measures of affect in adult animals that were exposed to alcohol as adolescents. For example, AIE animals exhibited depression-like signs, such as reduced consumption of a sugar solution or increased immobility in a swim test.[35-37] Similarly, alcohol exposure during early to mid-adolescence was associated with reliable increases in social anxiety in adulthood.[38,39] Interestingly, this effect seems to be sex-specific and is only observed in males. Other studies in male rats after AIE have detected increases in general anxiety, as indicated by decreased time on the open arms (relative to time on the closed arms) of an elevated plus maze.[37,40,41] However, increases in general anxiety have not always been observed.[36,42]

It is challenging to distinguish disinhibition and anxiety in animal studies. For example, although the elevated plus maze test was developed and validated as a test of anxiety, results from it are sometimes interpreted in terms of disinhibition. Increased time spent in an environment that animals perceive as more risky (i.e., the open arm of an elevated maze) could indicate either greater disinhibition, decreased anxiety, or some interaction of the two, with increases in disinhibition perhaps contributing to a suppression in anxiety.[30,34] In studies of adolescent alcohol exposure, AIE has been found to increase open-arm time in some studies, suggesting greater disinhibition, but to decrease open-arm time in others, a pattern of findings consistent with a profile of increased anxiety. It is possible that adolescent alcohol exposure can be characterized by profiles of both increased anxiety and disinhibition. Competition between these propensities—depending, for example, on the perceived stressfulness of the situation or the animals' previous handling—may explain these reliable but opposing outcomes.[43]

Retention of Adolescent Phenotypes Into Adulthood

One surprising long-lasting consequence of adolescent alcohol use observed repeatedly in AIE studies is the retention of adolescent phenotypes into adulthood. In rodent studies, adolescents have been shown to differ from adults in a variety of alcohol-related phenotypes. In instances where researchers could assess similar effects in human adolescents, the analyses uncovered comparable age-related differences.[2] For example, like their human counterparts, adolescent animals often voluntarily consume significantly more alcohol per drinking occasion than adults.[18,44,45] This elevated alcohol intake is particularly notable in male animals and mirrors intake by human adolescents.[46]

Adolescents often differ from adults in their sensitivity to alcohol's effects, with the direction of these differences dependent on the effect studied. Adolescents are less sensitive to many of alcohol's undesired effects, such as alcohol-induced motor impairment, sedation, aversion, and social impairment, which normally serve as cues to limit intake.[47] Adolescents are also less sensitive to acute withdrawal (i.e., hangover effects) after moderate to high alcohol consumption. In animal models, this effect has been reflected in reduced levels of withdrawal-associated anxiety.[48,49] In contrast to the attenuated sensitivity of adolescents to many of alcohol's undesired effects, adolescents are often more sensitive to certain desired effects of alcohol, such as its rewarding and social facilitating effects.[47] Adolescents are also usually sensitive to the disruptive effects of acute alcohol intoxication on learning and memory.[25] Collectively, adolescent-associated attenuated sensitivity to aversive effects and increased sensitivity to desirable effects of alcohol could contribute to enhanced susceptibility to the initiation and escalation of alcohol use during adolescence,[47] with intoxication having pronounced disruptive effects on learning and memory.[25]

Animals given repeated alcohol exposure during adolescence often retain adolescent-typical phenotypes into adulthood.[50] This persistence can be observed through baseline behavioral, cognitive, electrophysiological, and neuroanatomical assessments, as well as in the animals' responses to alcohol challenges in adulthood.[51] For example, animals exposed to alcohol during adolescence maintained an enhanced sensitivity to alcohol's rewarding and stimulatory effects into adulthood.[38,52-54] This persistent sensitivity could promote alcohol consumption in adulthood. In other studies, animals that experienced AIE retained their adolescent-typical insensitivities to alcohol's sedative, motor-impairing, and aversive effects, which could permit the maintenance of elevated alcohol drinking during adulthood.[53,55-58] Also, the decline in sensitivity to alcohol-induced deficits in spatial working memory that normally occurs between adolescence and adulthood did not occur in animals exposed to alcohol in adolescence.[59] As a result, adult animals exposed to AIE retain adolescent-like vulnerability to alcohol-induced memory impairments and show more memory disruption under the influence of alcohol than adults without a history of adolescent alcohol exposure.

Generally, retention of these adolescent phenotypes into adulthood is associated with alcohol exposure during adolescence; equivalent alcohol exposure during adulthood does not induce similar effects.[55,58] Moreover,

adolescent phenotypes are more pronounced if adolescent alcohol exposure is episodic, rather than continuous, reflecting typical adolescent binge-drinking consumption patterns.[55] An episodic exposure pattern can result in withdrawal episodes following each exposure, which could result in escalating withdrawal signs (e.g., increased anxiety-like behavior, lower seizure threshold, and more severe seizures), particularly in adolescents.[60,61]

Researchers are trying to uncover the neurobiological mechanisms that underlie the retention of adolescent phenotypes after adolescent alcohol exposure. One line of investigation has explored whether animals exposed to AIE retain into adulthood an immature balance of enhanced excitation to inhibition in the brain. Some analyses have assessed the role of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain. Studies found that in the hippocampus, inhibitory effects of GABA responsible for baseline levels of tonic inhibition normally are attenuated in adolescents; however, after AIE, this attenuation is maintained into adulthood.[50,62] Ethanol potentiation of this tonic inhibition is more marked in adolescents than adults—an effect that is maintained into adulthood after AIE.[50,62,63] These adolescent-typical neurophysiological characteristics and their persistence into adulthood may contribute to alcohol's enhanced memory-impairing effects in adolescents and to long-lasting memory impairment seen in adulthood after AIE.[51] More work is needed to identify the overall prevalence of persistent adolescent-typical immaturities after adolescent alcohol exposure under various baseline and challenge conditions, and to further characterize the mechanisms underlying these persisting effects.

Effects on Later Ethanol Consumption

Another potential long-term consequence of adolescent alcohol exposure that may reflect the persistence of adolescent phenotypes is elevated alcohol consumption during adulthood. Findings are mixed as to whether adolescent alcohol exposure increases adult alcohol consumption. The hypothesis is supported by findings that alcohol-preferring rats given free access to ethanol in their home cages throughout adolescence acquired an operant self-administration task for alcohol in adulthood more quickly than animals that did not have access to alcohol during adolescence.[64,65] Moreover, these animals exhibited greater resistance to extinction of the operant task, more spontaneous recovery of self-administration, and elevated response levels during reacquisition of the operant task compared with animals with no history of alcohol exposure. Similar findings were obtained in mice. Animals that had voluntary access to alcohol throughout adolescence consumed more alcohol as adults than mice whose access to alcohol was delayed until adulthood.[66] Rats exposed to alcohol through intermittent intraperitoneal administration in early to mid-adolescence later exhibited increased alcohol consumption, an effect that was not apparent when alcohol exposure was delayed until late adolescence.[41,67]

The findings of increased adult consumption levels following adolescent exposure are not universal, however. In some studies, adolescent rats exposed to alcohol vapor, and mice or rats given free access to alcohol in their home cages did not exhibit increased alcohol consumption during adulthood.[44,68,69] Other researchers found that animals given free access during adolescence to alcohol through an operant task demonstrated no increased operant response during adulthood, although they did show increases in some alcohol-related responses.[30,42]

Several variables may influence whether adolescent alcohol exposure increases adult alcohol consumption, which may explain the diverse findings. These variables include the sex of the animals, genetic background (i.e., the strain of rats or mice used), amount and mode of adolescent alcohol exposure, and assessment method of adult alcohol intake.[43] Also, when adolescent rats were given either a sweetened alcohol solution or the sweetened solution without alcohol, both groups later increased intake only of the solution they were exposed to during adolescence, not the alternate solution.[70] This suggests that increased alcohol intake during adulthood after consuming alcohol during adolescence may reflect increased acceptability of a familiar solution, rather than alcohol-specific effects. Although the existing data suggest that in some cases adolescent alcohol exposure can lead to increased consumption during adulthood, researchers still need to further clarify the circumstances in which these intake-enhancing effects emerge.

Neural Alterations

Alcohol exposure during adolescence has detrimental and potentially long-lasting effects not only on cognition, affect, and behavior, including future alcohol consumption, but also on the structure and function of the brain. Particularly pronounced effects include reductions in the formation of new brain cells (i.e., neurogenesis), long-lasting neuroinflammation, changes in gene expression through epigenetic mechanisms, and alterations in the activities of neurotransmitter systems in several vulnerable brain regions.

Neurogenesis and Cell Death

Adolescence is associated with a variety of neuroanatomical changes, including enhanced neurogenesis in some brain regions (e.g., the hippocampus).[71] Reductions in the numbers of neurons and in the connections between neurons (a process known as pruning) may occur in other regions of the brain (e.g., the PFC).[72] One of the most consistent neurological findings associated with adolescent alcohol ex-

posure is a reduction in neurogenesis and a region-specific increase in cell death and cell damage in the brain. The regions most commonly affected include the frontal cortex, hippocampus, amygdala, NAc, and cerebellum—regions that also undergo significant developmental changes during adolescence.[71,73-75] The adolescent brain seems to be particularly vulnerable to the effects of alcohol exposure because similar disruptions were not observed after equivalent exposure in adulthood.[73] The effects of binge-like exposure during adulthood occurred in different regions of the brain and were less pronounced than the effects of exposure during adolescence.[74]

Adolescent alcohol exposure affects not only the overall number of brain cells in specific brain regions but also their connections with each other. Recent studies investigated the effects of AIE on the structure and function of synapses in the hippocampus, a brain region associated with learning and memory.[76] The analyses found that AIE resulted in a greater proportion of immature relative to mature dendritic spines (specialized sites on neurons that receive and amplify input from signal-emitting neurons) in the brains of AIE animals compared with those of nonexposed adult animals. Animals with AIE also manifested more robust long-term potentiation as adults when they were compared with nonexposed animals, a pattern of neurophysiological activation similar to the pattern normally seen in adolescents. Long-term potentiation is the strengthening of synaptic connections when the synapses are repeatedly activated. Although this process is necessary for learning, greater than normal long-term potentiation has been linked to memory deficits and other learning-related behavioral changes.[76]

Neuroinflammation

Adolescent alcohol exposure has been shown to induce long-term increases in expression of several neuroimmune genes that encode proinflammatory signaling molecules.[77] Adolescent exposure also has been shown to activate Toll-like receptor 4 (TLR4), a receptor in the innate immune system that plays a central role in initiating innate immune responses throughout the body.[77] Ethanol-induced TLR4 activation triggers the expression of various transcription factors that, in turn, promote the expression of proinflammatory cytokines and other mediators of inflammation. In the short term, such proinflammatory responses may be adaptive. However, when these responses are maintained over longer periods, the result is long-lasting neuroinflammation.

In the brain, ethanol-induced activation of TLR4 and its subsequent actions can contribute to brain damage associated with excessive alcohol exposure.[77] For example, in animal studies, activation of TLR4 using a bacterial compound (i.e., lipopolysaccharide) induced a long-lasting reduction in neurogenesis similar to that observed after AIE.[71] In mice that did not produce TLR4, adolescent alcohol exposure did not result in the characteristic inflammatory, cognitive, and behavioral consequences usually associated with this exposure.[40,77]

The role of TLR4 and neuroinflammation in the functional and neural consequences of adolescent alcohol exposure is supported by findings that treatment with an anti-inflammatory compound (i.e., indomethacin) prevented the typical cell death and behavioral deficits seen after AIE.[28] These observations suggest that anti-inflammatory agents may represent a new class of pharmacotherapeutic interventions for preventing, ameliorating, or even reversing some of the long-term consequences of adolescent alcohol exposure.

Epigenetic Mechanisms

Adolescent alcohol exposure also influences gene expression by modifying epigenetic regulatory mechanisms. Adolescent animals exposed to alcohol show alterations in histone acetylation, which, in turn, influences DNA methylation and the level of gene expression.[41,78,79] Such epigenetic alterations have been identified in the amygdala, NAc, and PFC, which are brain structures involved in memory processing, decision-making, and emotional reactions. For example, rats with AIE exhibited persistent increases in histone deacetylation and reductions in histone acetylation in the amygdala,[41] resulting in reduced expression of certain genes (e.g., brain-derived neurotrophic factor [BDNF]). When the alcohol-induced deacetylation was prevented by treatment with a histone deacetylase inhibitor, histone acetylation levels in the amygdala normalized, and the transcription of BDNF was restored.[41] The effects of AIE on histone acetylation levels also may contribute to observed behavioral and neural effects of AIE. Treatment with the deacetylase inhibitor attenuated anxiety-like behaviors, reversed the increase in alcohol intake during adulthood, and normalized the decline in neurogenesis usually exhibited by AIE animals.[41,80]

Neurotransmitter Systems

Alcohol exerts its dose-dependent and region-specific effects largely through direct or indirect interactions with the major neurotransmitter and neuromodulatory systems in the brain, including the GABA system discussed earlier, as well as the dopamine, serotonin, glutamate, acetylcholine, and endocannabinoid systems.[81] However, there is specificity in these effects, and not all systems and brain regions are equally vulnerable. Many of these alcohol-sensitive neurotransmitter and neuromodulatory systems and affected brain regions undergo developmental transformations during adolescence, and they may be especially vulnerable to alcohol-induced perturbations during development. Indeed, AIE has been shown to be associated with alterations in several of these systems, including:

- **Changes in the activity of the dopamine system in the NAc.** Several studies have reported enhanced dopamine function in neurons projecting to the NAc, a pivotal component of the brain's reward system, following AIE. These neurons exhibited increased dopamine-mediated neurotransmission under normal conditions and after an alcohol challenge.[78,82,83] The neurons also exhibited higher basal extracellular dopamine levels.[78,84] Given the critical role that dopamine plays in facilitating reward-related motivation and behaviors, these findings suggest that AIE may enhance the rewarding experiences associated with alcohol, which could promote further alcohol ingestion.
- **Changes in the activity of the glutamate system.** Glutamate is the primary excitatory neurotransmitter in the brain and acts via several types of receptors, including the N-methyl-D-aspartate (NMDA) receptor. AIE has been reported to increase NMDA receptor binding in the frontal cortex, as well as the expression of one subunit of this receptor (i.e., the NR2B subunit).[85] Other research has reported a decrease in the subunit's phosphorylation.[78] Altered NMDA functioning in the PFC has been suggested to disrupt functioning of that brain region and to contribute to the impulsive behavior and the lack of control over drinking that is characteristic of individuals with AUD.[78]
- **Changes in the acetylcholine system in the basal forebrain.** One reliable consequence of AIE observed in rodent studies is a long-lasting decrease in the basal forebrain of the number of neurons that exhibit activity of the choline acetyltransferase enzyme, which is required for synthesis of the neurotransmitter acetylcholine. This effect is seen following adolescent, but not adult, alcohol exposure.[29,31,36,86] These findings suggest that adolescent alcohol exposure impairs the normal cholinergic neurotransmission in the basal forebrain that is crucial for ensuring cortical plasticity and learning. Hence, AIE-induced deficits in the cholinergic system may contribute to future cognitive deficits.

Repeated alcohol use during adolescence induces specific alterations in a variety of neural systems that play critical roles in neural, cognitive, and behavioral function. It is possible that some of these neural alterations reflect positive adaptations to AIE to mitigate long-term consequences of the alcohol exposure. Yet, these potential compensations do not appear to be sufficient, given the growing list of long-term consequences of AIE on later neurocognitive and behavioral function.

Conclusions and Future Directions

Adolescence is characterized by social and emotional development and often is accompanied by experimentation with AODs. Brain development continues during adolescence, and, increasingly, adolescence is being viewed as a period of enhanced brain plasticity and experience-related brain sculpting. Many adolescent experiences (e.g., education, sports, and positive social interactions) provide beneficial long-term sculpting. Other influences, such as repeated exposure to alcohol, can be detrimental and have long-term effects on neural functioning, cognition, and behavior, including enhanced AOD consumption, that persist into adulthood.

Studies conducted primarily using rodent models of adolescence have shown that propensity for the initiation and escalation of alcohol use during adolescence may be promoted by adolescents' greater sensitivity to the socially facilitating and rewarding effects of alcohol, combined with a reduced sensitivity to other effects (e.g., social and motor impairment, and sedative and aversive effects) that likely serve as cues to terminate intake. Animal studies have shown that repeated exposure to alcohol during adolescence, especially AIE that mirrors binge-drinking patterns observed in human adolescents, induces specific patterns of sustained neurobehavioral alterations that may promote further drinking. Particularly worrisome are reports that adolescent alcohol exposure may lead to the retention of adolescent phenotypes—including adolescent-typical responses to alcohol—into adulthood. Other cognitive, behavioral, and affective consequences have been reported after AIE, including impaired performance of executive functions, memory impairment, reduced cognitive flexibility, greater risk preference and disinhibition, and elevated social (and sometimes general) anxiety. In many cases these effects are specific to adolescent alcohol exposure and are not evident after equivalent alcohol exposure during adulthood.

Animal studies also have identified lasting neural alterations induced by AIE that may contribute to behavioral and cognitive changes. These changes include reduced neurogenesis, increased neuroinflammation, epigenetic alterations, and alterations in numerous neurotransmitter systems, including glutamate, GABA, the balance between these excitatory and inhibitory systems, dopamine, and the basal forebrain cholinergic system. When different age groups were compared, the consequences typically were more pronounced after adolescent alcohol exposure than after equivalent adult exposure. Likely anatomical targets for these long-term effects include the hippocampus, amygdala, NAc, and PFC. These neural systems underlie the developmental shifts in sensitivity to drug rewards and drug aversion that normally occur during adolescence and adulthood. These systems are also involved in neurodevelopmental processes related to socioemotional

functioning and advanced aspects of cognitive functioning.

Despite the progress achieved using animal models for understanding the consequences of adolescent alcohol exposure and, particularly, the intermittent, binge-like exposures characteristic of this age, many questions remain. For example, additional research is needed to elucidate how AIE affects the neural mechanisms underlying the enhanced reward and attenuated aversive sensitivities that are normally seen during adolescence and are maintained into adulthood after AIE, as well as how these mechanisms contribute to later alcohol consumption. It also will be crucial to determine if lasting functional consequences of adolescent alcohol exposure can be prevented, attenuated, or reversed by blocking alcohol-induced neural alterations. Similarly, researchers need to further elucidate the persistence of adolescent phenotypes into adulthood that has been reported after adolescent alcohol exposure. The breadth and limitations of this adolescent-like persistence across different functional domains, its stability over time, and whether it can be reversed or modified all need to be examined. It is undoubtedly useful and necessary to use animal models to study contributors to and consequences of adolescent-typical behaviors such as alcohol consumption. Nonetheless, the findings are only useful if they prove valid, applicable to predicting the effects of adolescent alcohol exposure in humans, and ultimately relevant to prevention and treatment.

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Alcohol's Effects on the Brain: Neuroimaging Results in Humans and Animal Models

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Brain imaging technology has allowed researchers to conduct rigorous studies of the dynamic course of alcoholism through periods of drinking, sobriety, and relapse and to gain insights into the effects of chronic alcoholism on the human brain. Magnetic resonance imaging (MRI) studies have distinguished alcohol-related brain effects that are permanent from those that are reversible with abstinence. In support of post-mortem neuropathological studies showing degeneration of white matter, MRI studies have shown a specific vulnerability of white matter to chronic alcohol exposure. Such studies have demonstrated white-matter volume deficits as well as damage to selective gray-matter structures. Diffusion tensor imaging (DTI), by permitting microstructural characterization of white matter, has extended MRI findings in alcoholics. MR spectroscopy (MRS) allows quantification of several metabolites that shed light on brain biochemical alterations caused by alcoholism. This article focuses on MRI, DTI, and MRS findings in neurological disorders that commonly co-occur with alcoholism, including Wernicke's encephalopathy, Korsakoff's syndrome, and hepatic encephalopathy. Also reviewed are neuroimaging findings in animal models of alcoholism and related neurological disorders. This report also suggests that the dynamic course of alcoholism presents a unique opportunity to examine brain structural and functional repair and recovery.

Key words: Magnetic Resonance Imaging; Diffusion Tensor Imaging; MR Spectroscopy; alcohol use disorder; thiamine; liver; cerebellum

Apart from direct effects on the brain, excessive alcohol consumption is associated with increased risk for trauma (i.e., traumatic brain injury) (Alterman and Tarter 1985; Chen et al. 2012), seizures (Eyer et al. 2011; Martindale et al. 2011), and stroke (de los Rios et al. 2012; Suzuki and Izumi 2013), each of which can have effects on brain structure independent of alcohol or each other. Furthermore, alcohol can alter the brain by affecting peripheral organs, including the digestive tract (e.g., Bienia et al. 2002; Duell et al. 2012), liver (e.g., Cederbaum 2012), heart (e.g., Roerecke et al. 2011), pancreas (e.g., Andersen et al. 2008), kidneys (e.g., Schaeffner and Ritz 2012), and lungs (e.g., Yeligar et al. 2012). Mechanisms of these indirect effects of alcohol on the brain are likely mediated via soluble factors, such as ceramides (e.g., de la Monte et al. 2012).

To evaluate alcohol's central nervous system effects, researchers distinguish "uncomplicated alcoholism" (i.e., alcohol use disorder [AUD]) from the various clinically diagnosable consequences of chronic alcohol consumption, including Wernicke's encephalopathy (WE), Korsakoff's syndrome (KS), hepatic encephalopathy (HE), central pontine myelinolysis (CPM), alcoholic cerebellar degeneration (ACD), alcohol-related dementia (ARD), and Marchiafava-Bignami disease (MBD).[1] The use of brain-imaging technology to evaluate clinically defined syndromes associated with chronic alcoholism, each with relatively unique radiological signatures (see table 1 and figure 1), provides guideposts for studying brain alterations associated with uncomplicated alcoholism.

Approximately 7 percent of adults age 18 and older have an AUD (Substance Abuse and Mental Health Services Administration 2013).

[1] WE and KS are neurological disorders caused by thiamine deficiency. HE is a decline in brain function as a result of severe liver disease. CPM is a neurological disorder resulting from the destruction of the myelin layer that covers nerve cells in the pons. ACD occurs when neurons in the cerebellum are damaged due to alcohol use. MBD is a neurological disease associated with alcoholism, caused by damage to the corpus callosum.

Prevalence estimates of alcoholism-related syndromes are difficult to ascertain. Incidence estimates often are based on postmortem findings. Postmortem evaluation indicates a prevalence of 2 percent of WE in the general population; however, as many as 12 to 18 percent of alcoholics can have postmortem evidence of WE (Harper et al. 1988; Riethdorf et al. 1991; Thomson et al. 2002). Based on observations that 80 to 85 percent of patients with WE can develop KS, the estimated prevalence of KS is 11 to 12 percent of the alcoholic population (Day et al. 2013; Victor et al. 1971). Wernicke-Korsakoff syndrome (WKS) is used to refer to the presence of both WE and KS because of the close relationship between the two disorders.

Estimates of HE are derived from estimates of alcoholic cirrhosis, which can range from 8 percent to 20 percent (Bellentani et al. 1997; Mann et al. 2003; Sorensen et al. 1984). Mild HE occurs in up to 80 percent of cirrhotic patients, and overt HE occurs in up to 45 percent of cirrhotic patients (Bajaj 2008; Poordad 2007). One study estimated the incidence of CPM at 0.5 percent among the general population (Newell and Kleinschmidt-DeMasters 1996). However, prevalence is much higher (30 percent) among patients with liver transplants (Singh et al. 1994). For ACD prevalence, reports based on postmortem evaluation range from as low as 0.4 percent to as high as 42 percent of alcoholics (Riethdorf et al. 1991; Scholz et al. 1986; Stork 1967; Torvik and Torp 1986). Rates of ARD can depend on the setting, with facilities specializing in early identification and treatment of memory disorders reporting rates of 3 percent (McMurtray et al. 2006) and nursing homes reporting rates as high as 24 percent (Carlen et al. 1994; Oslin and Cary 2003; Ritchie and Villebrun 2008). Prevalence can also depend on the age of the population evaluated (i.e., higher prevalence of ARD is found in younger- onset [i.e., ages 45–64] dementia) (Draper et al. 2011*b*; Harvey et al. 2003). MBD appears to be very rare, with only about 250 cases reported between 1966 and 2001 (Helenius et al. 2001).

Human studies offer a full depiction of the consequences of chronic alcohol exposure but are limited by ethical considerations. That is, rigorous experimentation requires the ability to control for relevant variables such as the premorbid condition of the brain. The wide variation (or heterogeneity) of alcoholic populations examined with respect to genetic predisposition, age of onset, pattern of drinking, frequency of withdrawals, length of sobriety, nutritional, and hepatic status has hampered researcher attempts to isolate specific brain regions and mechanisms affected by alcohol, per se. This heterogeneity, and the complexity that it introduces, makes it difficult to thoroughly characterize the disorder. Animal models, in contrast to the indefinite natural course of alcohol use in humans, allow researchers to determine alcohol toxicity in a way that allows them to control for multiple genetic, environmental, and alcohol consumption factors. Animal models permit the study

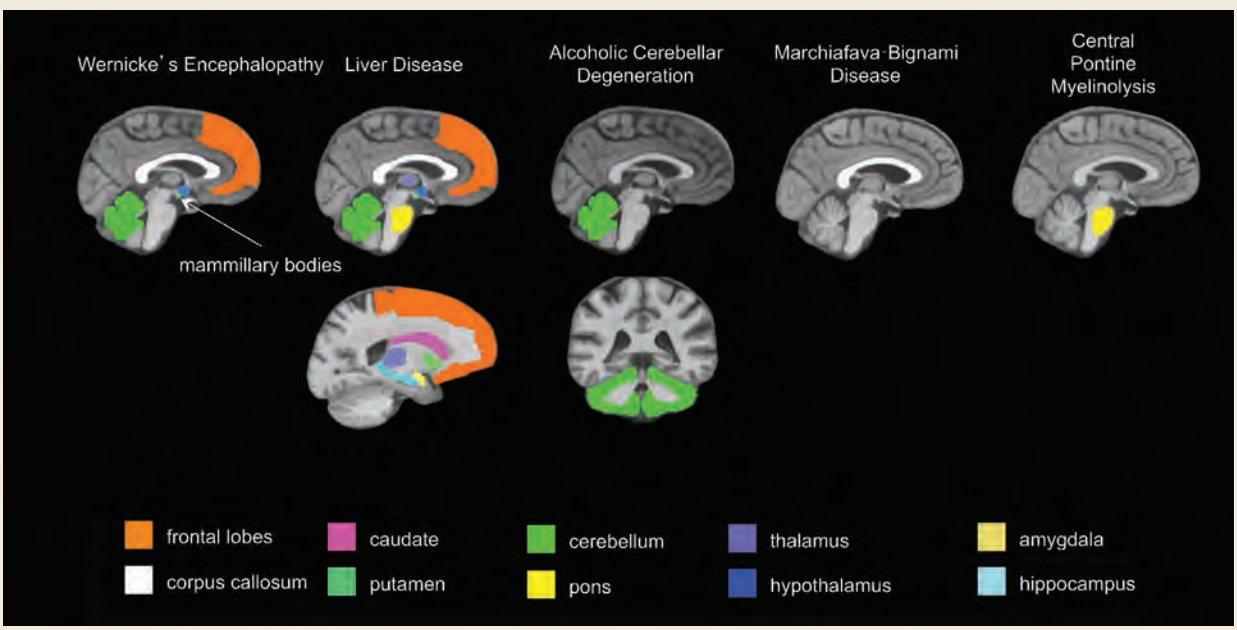

Figure 1 Brain regions targeted by alcoholism-related diseases.

of underlying mechanisms, enabling researchers to better interpret findings from human studies.

On the other hand, animal models also have limitations. Species differences in brain structure and function—among myriad other differences between humans and other animals—can give inadequate information when animal data are applied to human disease. For example, mice models fail to mimic human inflammatory disease with respect to genomic responses (Seok et al. 2013), and corticosteroids disturb development in animals but not in humans (Needs and Brooks 1985). Furthermore, researchers have hypothesized that the design, conduct, and analysis of a mainstay of animal experiments are questionable (Matthews 2008) and rarely undergo meta-analytical review for consensus (Mignini and Khan 2006; Peters et al. 2006; Pound et al. 2004; Sandercock and Roberts 2002).

This article reports key findings in humans, from macrostructural findings using magnetic resonance imaging (MRI), microstructural findings using diffusion tensor imaging (DTI), and metabolic findings from MR spectroscopy (MRS). Studies of alcohol-related central nervous system disorders are used as a framework for findings in uncomplicated alcoholism. The article also examines studies of abstinence and relapse and current imaging studies of animal models of alcoholism and co-occurring brain disorders. The evidence suggests that human studies are necessary to identify and classify the brain systems modified by concomitants of alcoholism versus alcoholism, per se, and that animal models of alcoholism and its co-occurring brain disorders are essential for a mechanistic understanding of vulnerable brain systems.

Structural MRI

Since the early 1980s, conventional structural MRI has allowed researchers to visualize the living human brain. Detailed images of the brain are possible in part because the different brain tissue types (i.e., gray matter, white matter, and cerebrospinal fluid [CSF]) contain different proportions of water (Rumboldt et al. 2010). With MRI, the brain can be viewed from bottom to top (axial), from front to back (coronal), from left to right (sagittal), or at any oblique angle to these planes. This flexibility also enables greater accuracy in aligning images with internal landmarks, an essential consideration for ensuring consistency of data from replicate images from the same individual (Rohlfing 2006).

Structural MRI Findings in Alcoholism-Related Brain Diseases

Wernicke-Korsakoff Syndrome

WE occurs with chronic alcoholism and thiamine deficiency. If untreated, WE patients can develop KS, a severe neurological disorder characterized by anterograde amnesia (Harper 2006; Zahr et al. 2011). Malnutrition, vomiting, and diarrhea are common in chronic alcoholism and can contribute to thiamine deficiency (Fields et al. 1994; Gloria et al. 1997; Morgan 1982; Ross et al. 2012). Further, the gastrointestinal tract's ability to absorb necessary quantities of thiamine is diminished in alcoholics (Hoyumpa 1980; Thomson 2000), and the liver, which houses a large part of the body's supplies of thiamine, may not be able to store thiamine in the same capacity if it is in a diseased state (Butterworth 2009; Levy et al. 2002). Classical clinical signs of WE included visual, gait, and mental disturbances (Victor et al. 1971), but more recent assessments describe mild, moderate, and severe signs and symptoms including anorexia, loss of memory, and emotional changes (Thomson et al. 2008). An MRI image of acute WE (see figure 2) has symmetrical bright spots, or hyperintensities,

Table 1 Radiological Signatures in Brain Imaging of Patients with Alcoholism-Related Syndromes

Alcoholism-Related Syndrome	Abbreviation	Primary Targeted Region(s)	Secondary Targeted Regions	Prevalence in Alcoholics (Percentage)
Wernicke's Encephalopathy	WE	Mammillary bodies, periaqueductal gray matter, dorsal medulla, tectal plates, olivary bodies, pons, tissue surrounding 3rd ventricle		12–18
Korsakoff's Syndrome	KS	Mammillary bodies, hippocampus, thalamus, orbitofrontal cortices	Cerebellum, pons	10–15
Hepatic Encephalopathy	HE	Globus pallidus, substantia nigra	Corticospinal tract, cortex	3–16
Central Pontine Myelinolysis	CPM	Pons	Basal ganglia, thalamus, cerebral gray–white matter junctions	< 0.5
Alcoholic Cerebellar Degeneration	ACD	Cerebellum		0.4–42
Alcohol-Related Dementia	ARD	Frontal cortex		3–24
Marchiafava-Bignami Disease	MBD	Corpus callosum	Cortex	< 0.002

clearly visible on T2-weighted images, and those created by fluid attenuation inversion recovery[2] (FLAIR). The bright spots appear in the midbrain gray matter surrounding the cerebral aqueduct (i.e., periaqueductal gray matter), mammillary bodies, and tissue surrounding the third ventricle[3] (Lenz et al. 2002; Sullivan and Pfefferbaum 2009). These findings agree with postmortem diagnosis of WE, often requiring evidence of lesions in the mammillary bodies and periventricular areas (e.g., Caine et al. 1997). In addition, observed MR hyperintense areas in WE include the thalamus, cerebellar vermis (Murata et al. 2001), dorsal medulla, tectal plates (Ha et al. 2012), olivary bodies, and dorsal pons (Liou et al. 2012). MRI analysis of KS patients compared with unaffected research participants (i.e., nonalcoholic control subjects) revealed substantial volume shrinkage of the mammillary bodies in KS and a lesser but significant volume deficit in uncomplicated alcoholics (Sheedy et al. 1999; Sullivan et al. 1999*b*; but see Shear et al. 1996; Victor et al. 1989). In contrast with early MR studies suggesting that KS affects the mammillary bodies while sparing the hippocampi (Squire et al. 1990), more recent work demonstrates hippocampal volume deficits in KS (Sullivan and Marsh 2003). Other regions affected by KS are the thalamus, orbitofrontal cortex (Jernigan et al. 1991*b*), cerebellum, and pons (Zahr et al. 2009).

Hepatic Encephalopathy (HE)

HE, occurring in acute or chronic liver disease, including acute liver failure and cirrhosis, is believed to arise, at least partially, from high levels of ammonia circulating in the blood. HE patients may appear confused and disoriented and have poor coordination (Prakash and Mullen 2010; Vaquero et al. 2003). T1-weighted images of HE show bilateral, symmetrical, and high-intensity signals in basal ganglia structures, particularly the globus pallidus and substantia nigra (Binesh et al. 2006; Cordoba et al. 2002; Naegele et al. 2000; Pujol et al. 1996; Taylor-Robinson et al. 1995) (see figure 3). T2-weighted FLAIR images show hyperintense signals along the corticospinal tract and diffuse increases in white-matter signal intensities in the cerebral hemispheres (Rovira et al. 2002, 2008). These in vivo MR features correspond with evidence of increased numbers of nonneuronal (i.e., glial) cells called astrocytes in basal ganglia and cerebral cortex of HE brains (Caine et al. 1997). Although discriminating features of WE and HE have been outlined, these diseases can be difficult to differentially diagnose and distinguish, because patients can appear to have similar symptoms and comparable MRI results, especially among alcoholics (Thorarinsson et al. 2011).

[2] Researchers use different MRI techniques to highlight different aspects of the brain. Techniques mentioned in this article include T1 weighted, T2 weighted, and FLAIR.

[3] The cerebral aqueduct and third ventricle are part of the brain's ventricular system—a set of cavities in the brain that produce, transport, and remove cerebrospinal fluid. This system also includes the lateral ventricles and fourth ventricle.

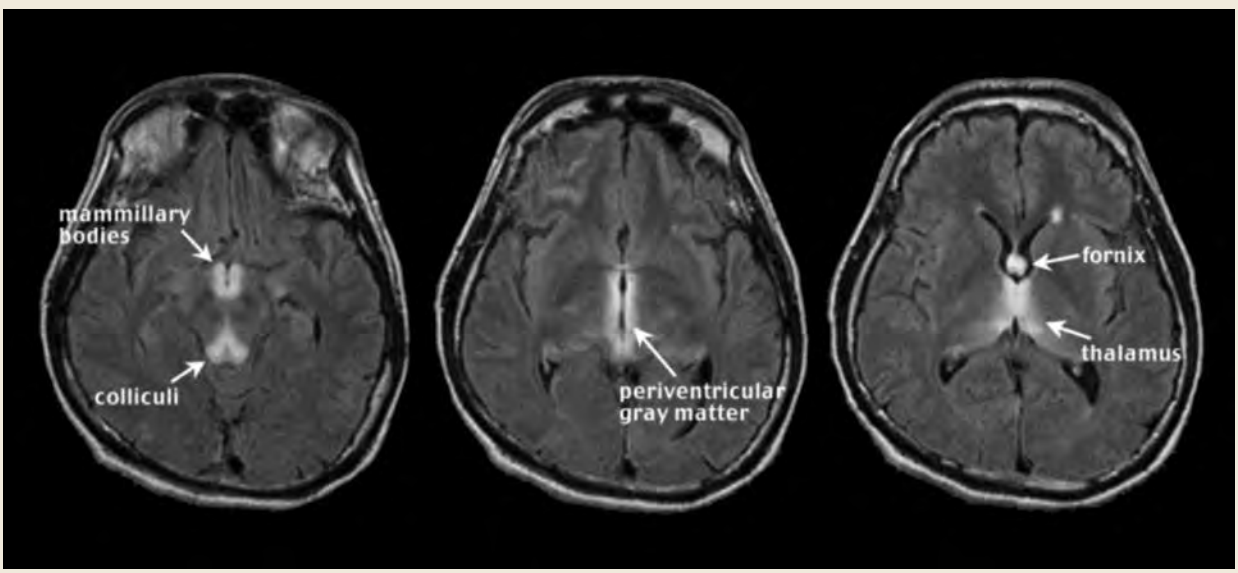

Figure 2 Wernick's encephalopathy (WE). In acute WE, magnetic resonance imaging (MRI) can detect symmetrical, bilateral hyperintense foci, visible on T2-weighted and fluid attenuation inversion recovery (FLAIR) images, in periaqueductal gray matter, mammillary bodies, and tissue surrounding the third ventricle.

Central Pontine Myelinolysis (CPM)

A significant proportion of CPM cases (Goebel and Herman-Ben Zur 1976; Messert et al. 1979) include a history of alcoholism. People with CPM, which is associated with electrolyte disturbances and specifically with aggressive correction of low sodium levels in the blood (i.e., hyponatraemia) (Chua et al. 2002), may have symptoms such as the inability to control facial movements, decreased voluntary muscle control (i.e., ataxia), and acute changes in consciousness (Kumar et al. 2006; Pfister et al. 1985). Classically, CPM was characterized by the presence of a symmetric triangular or "bat-wing" lesion in the pons (DeWitt et al. 1984; Gerard et al. 1987), with hypointense T1-weighted (Kleinschmidt-Demasters et al. 2006; Martin and Young 1995) and hyperintense T2-weighted (Buis and Wijdicks 2002; Kleinschmidt-Demasters et al. 2006; Pfister et al. 1985; Martin and Young 1995) images (see figure 4) reflecting damage to the protective covering of nerve cells (i.e., demyelination) as noted postmortem (Goldman and Horoupian 1981). The term osmotic myelinolysis (e.g., Chua et al. 2002; de Souza and Desai 2012) was coined to reflect the fact that other brain regions (e.g., basal ganglia, thalami, and cerebral gray–white matter junctions) are affected in CPM (e.g., Chen et al. 1996; Graff-Radford et al. 2011; Hagiwara et al. 2008; Harlan et al. 1988; Price et al. 1987; Waragai and Satoh 1998), despite suggestions that pathology in these other regions may not strictly represent demyelination (Kleinschmidt-Demasters et al. 2006; Kumar et al. 2006). Because a postmortem study of 112 autopsy cases of CPM patients reported that 28 percent could also be diagnosed with WE (Goebel and Herman-Ben Zur 1976), pontine dysfunction should be regarded as a cardinal clinical sign of CPM.

Alcoholic Cerebellar Degeneration (ACD)

ACD patients most frequently display ataxia, although other symptoms can include uncontrollable and repetitive eye movement (i.e., nystagmus) and speech problems resulting from impaired muscle control (i.e., dysarthria) (Fitzpatrick et al. 2012). Neuroimaging in ACD demonstrates damage disproportionately apparent in anterior superior portions of the cerebellar vermis (Sullivan et al. 2000*a*), with postmortem pathology indicating loss of cerebellar Purkinje cells (Feuerlein 1977).

Alcohol-Related Dementia (ARD)

Alcoholic dementia, or ARD, a currently preferred term, remains a controversial diagnosis because of confounding syndromes such as WE and HE. Nevertheless, certain clinically distinguishing features of ARD exist. It often occurs in socially isolated men at younger ages of onset (i.e.,

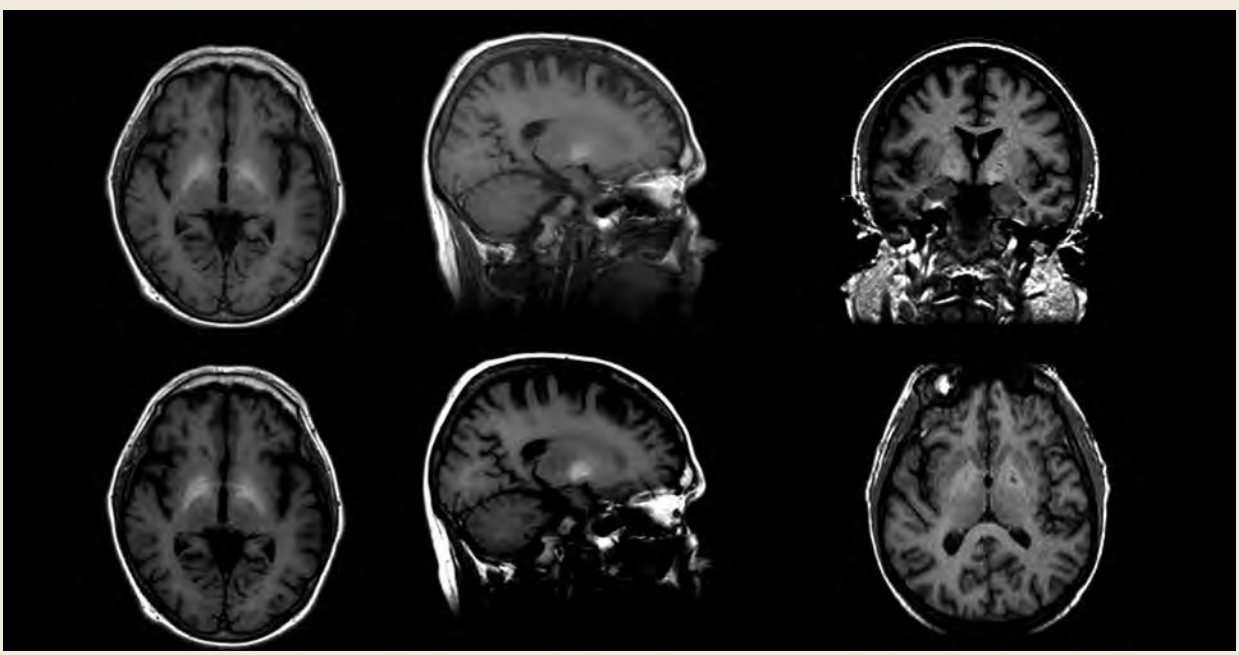

Figure 3 Hepatic encephalopathy (HE). T1-weighted imaging in HE reveals bilateral, symmetrical, high-intensity signals in basal ganglia structures, particularly the globus pallidus and substantia nigra, probably due to manganese deposition and T1 shortening. T2-weighted fluid attenuation inversion recovery (FLAIR) shows hyperintense signals along the corticospinal tract and diffuse hyperintense white matter signal in the cerebral hemispheres.

younger than age 65) than other types of dementia (Draper et al. 2011*a*; Ridley et al. 2013); deficits in visuospatial, executive, and memory functions (Schmidt et al. 2005); slower progression compared with other types of dementia (Gupta and Warner 2008); and partial reversibility (Oslin and Cary 2003). ARD is considered a frontal dementia (Stewart 2006). In support of such categorization, forensic evaluation of a sample of alcoholic brains noted a consistent pattern of synaptic loss in the superior laminae of the frontal cortex (i.e., Brodmann area 10), not related to liver disease (Brun and Andersson 2001).

Marchiafava-Bignami Disease (MBD)

MBD, a disease marked by mildly impaired mental status (e.g., confusion) and sometimes by dysarthria (Lee et al. 2011) or ataxia (Arbelaez et al. 2003), is poorly understood but may be related to nutritional deficiencies in addition to chronic alcohol consumption (Kawamura et al. 1985). Traditionally characterized by demyelination and necrosis of the corpus callosum, a number of reports identify cortical lesions in so-called MBD (Ihn et al. 2007; Johkura et al. 2005; Khaw and Heinrich 2006; Namekawa et al. 2013; Tuntiyatorn and Laothamatas 2008; Yoshizaki et al. 2010). Such data, however, represent single case studies and may reflect inaccurate MBD diagnoses. As observed in the pons in CPM, lesions (Clavier et al. 1986) appear hyperintense on T2-weighted images (Bano et al. 2009; Carrilho et al. 2013; Gambini et al. 2003) and hypointense on T1-weighted images (Bano et al. 2009; Carrilho et al. 2013; Kawamura et al. 1985) and often are located along the entire extent of the corpus callosum (Hillbom et al. 2014).

Given the aforementioned findings in clinically differential and diagnosable alcohol-related syndromes, the following section examines whether similar brain disorders also appear in alcoholics who do not manifest the full spectrum of symptoms present in these conditions. That is, how do the brains of uncomplicated alcoholics compare? Quantitative MRI has shown that relatively mild yet significant structural deficits characteristic of alcoholic syndromes can occur in uncomplicated alcoholics.

Structural MRI Findings in Uncomplicated Alcoholism

Relative to findings in WKS, research demonstrates mild volume deficits in the mammillary bodies (Shear et al. 1996; Sullivan et al. 1999), hippocampi, and thalami in uncomplicated alcoholics compared with healthy controls (De Bellis et al. 2005; Chanraud et al. 2007; Pitel et al. 2012; Sullivan 2003; van Holst et al. 2012). As shown in figure 5, these structures show a graded effect of volume deficits. That is, volume deficits are greatest in brains of subjects with KS (figure 5C) compared with brains of subjects with uncomplicated alcoholism (figure 5B) and brains unaffected by alcohol (figure 5A). Results suggest that mammillary-body damage is not prerequisite for the development of amnesia in alcoholism (Shear et al. 1996). MR findings also show hippocampal volume deficits in alcoholics compared with healthy controls (Agartz et al. 1999; Beresford et al. 2006; Kurth et al. 2004; Laakso et al. 2000; Sullivan et al. 1995; Wilhelm et al. 2008). Hippocampal volume deficits in alcoholism are influenced by age (Sullivan et al. 1995), even though

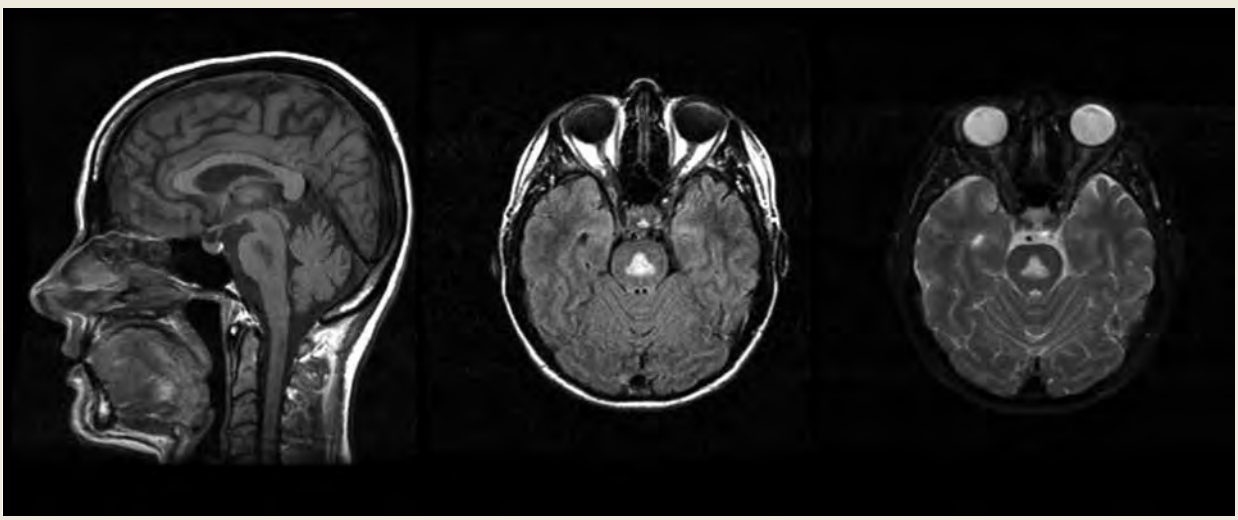

Figure 4 Central pontine myelinolysis (CPM) is visualized as a hypointense T1 (left, sagittal slice) or hyperintense T2 (middle, right axial slices are early and late echo images) symmetric triangle or "bat-wing" lesion in the pons.

age-related decline is difficult to detect in cross-sectional studies (Pfefferbaum et al. 2013; Raz et al. 2010; Sullivan et al. 2005*b*). Although deficits in hippocampal volume are not related to seizure incidence (Bleich et al. 2003; Sullivan et al. 1996), temporal-lobe white matter may be sensitive to alcohol-withdrawal seizures (Sullivan et al. 1996). Hippocampal volume shrinkage in alcoholism is attributed to loss of white matter and decreased axonal diameter (Harding et al. 1997). Glial cell loss (Korbo 1999) or reduced incorporation of newly formed neurons to the dentate gyrus (He et al. 2005; Nixon and Crews 2004), however, could also affect hippocampal volume in alcoholism.

Other regions selectively affected in WE and KS include the orbitofrontal cortices (KS), periaqueductal gray matter, and tissue surrounding the third ventricle (WE). Reports suggest that propensity to relapse following sobriety is related to pronounced atrophy in bilateral orbitofrontal cortices (Beck et al. 2012; Cardenas et al. 2011; Durazzo et al. 2011; also see Rando et al. 2011). The third ventricle (i.e., enlargement) is sensitive to resumption of chronic alcohol consumption (Pfefferbaum et al. 2001; Sullivan et al. 2000*b*). There currently are no studies regarding periaqueductal gray-matter volume in uncomplicated alcoholics.

Key regions affected in HE include the globus pallidus and substantia nigra. Volume effects on these two structures have not been reported in uncomplicated alcoholics; however, in children with fetal alcohol syndrome, globus pallidus volume is reduced in size compared with unaffected children (Nardelli et al. 2011). In contrast, other basal ganglia nodes of reward circuitry have been described as affected in uncomplicated alcoholism (Durazzo et al. 2011; Makris et al. 2008): MRI studies have revealed smaller volumes of caudate (Boutte et al. 2012), putamen (Jernigan et al. 1991*a*), amygdala (Fein et al. 2006), and nucleus accumbens, especially in more recently sober alcoholics compared with healthy controls (Sullivan et al. 2005*a*). Given the role of the amygdala in emotional regulation and behavioral control (for review, see McBride 2002), however, researchers have speculated that premorbid amygdala volume deficits put individuals at heightened risk for developing AUD (Benegal et al. 2007; Clarke et al. 2008; Kamarajan et al. 2006).

CPM targets the pons and ACD affects the cerebellum. Total infratentorial volume (including pons, cerebellar hemispheres, vermis, fissures, cisterns, and fourth ventricle) is significantly smaller in uncomplicated alcoholics than control subjects. The volume of the pons (Chanraud et al. 2009*b*; Pfefferbaum et al. 2002*b*; Sullivan 2003) and cerebellum (i.e., hemispheres) (Boutte et al. 2012; Chanraud et al. 2007, 2009*a*; De Bellis et al. 2005; Sullivan et al. 2000*a,c*) is smaller in uncomplicated alcoholics than in normal controls. Alcoholism-related volume deficits are also prevalent in gray and white matter (Shear et al. 1996; Sullivan et al. 2003) of the cerebellar vermis (Antunez et al. 1998; Piguet et al. 2006; Sullivan et al. 2006*b*, 2010), predominately in anterior superior but not posterior inferior regions (Sullivan et al. 2000*a*) (see figure 6).

The frontal cortex is notably damaged in ARD. With respect to cortical regions in uncomplicated alcoholism, various methods have shown significant,

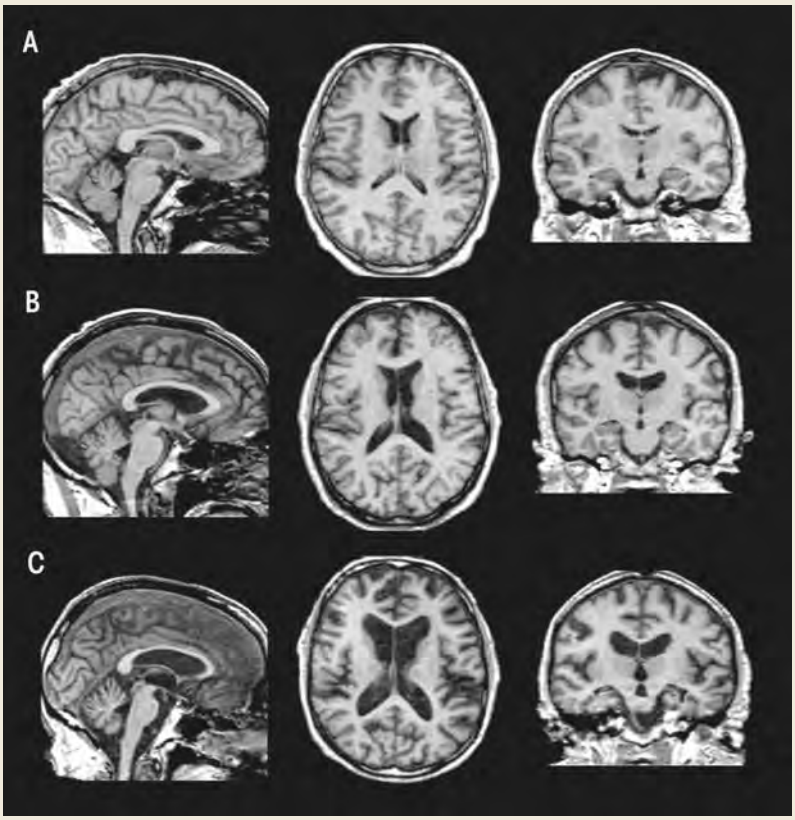

Figure 5 Brain volume deficits in a healthy control **(A)** compared with a subject with uncomplicated alcoholism **(B)** and a subject with Korsakoff's syndrome (KS) **(C)**. These structural MRIs show a graded effect of volume deficits, notable in the ventricular and sulcal cerebrospinal fluid (CSF)–filled spaces: subjects with KS > subjects with uncomplicated alcoholism > normal controls.

widespread shrinkage of both cortical gray and white matter with corresponding increases in CSF-filled spaces (Cardenas et al. 2007; Jang et al. 2007; Jernigan et al. 1991*a*; Mechtcheriakov et al. 2007; Pfefferbaum 1992). In particular, older (older than age 50) but not younger adult alcoholics show disproportionate deficits in both gray- and white-matter cortical volume, especially in the frontal lobes, when volumes are statistically adjusted for brain tissue decline associated with normal aging (Cardenas et al. 2005, 2007; Pfefferbaum et al. 1997). This is the case even in comparisons made in groups selected on alcohol consumption, where older alcoholics have consumed equivalent amounts over their lifetime as younger alcoholics.

Thinning of the corpus callosum occurs in uncomplicated alcoholics and is more prominent in the anterior than posterior regions (Estruch et al. 1997; Pfefferbaum et al. 1996). As with WE and KS, evidence for MBD-like pathology in uncomplicated alcoholism raises the possibility that brain damage occurs on a continuum. The following section examines how brain structures and function respond when drinking stops.

Structural MRI Findings in Recovery From Alcoholism

Longitudinal MRI investigations show that the ventricles become smaller following weeks (Schroth et al. 1988; Zipursky et al. 1989) or months (Shear et al. 1994) of drinking cessation. Reduction of lateral ventricles precedes reduction of third-ventricular volume (Pfefferbaum et al. 1995) and may be related to improvements in hematocrit, hemoglobin, and red blood cell counts (Pfefferbaum et al. 2004). The following brain structures increase in volume in response to abstinence: the entire cerebral cortex (Liu et al. 2000); temporal, insular, and anterior cingulate cortices (Cardenas et al. 2007); amygdala (Wrase et al. 2008) (a finding that would argue against a premorbid volume deficit); thalamus (Cardenas et al. 2007); hippocampus (Liu et al. 2000, Wrase et al. 2008); brainstem; and cerebellar cortex (Cardenas et al. 2007; Liu et al. 2000).

Sober alcoholics reveal several associations between brain-volume gain, as determined by MRI, and improvement in neuropsychological test performance: Reduced lateral-ventricle volume is related to improved memory performance (Rosenbloom et al. 2007), reduced third-ventricle volume is related to improved nonverbal short-term memory performance (Sullivan et al. 2000*b*), and reduced fourth-ventricle volume is related to improvement in measures of ataxia (Rosenbloom et al. 2007).

The brain's capacity to return to "normal" following long-term sobriety is unknown. Short-term (6 weeks) abstinence seems sufficient to observe some brain-volume recovery but does not result in equivalent brain volumes between recovering chronic alcoholics and healthy controls (Mann et al. 2005). It is difficult to determine whether recovery is complete. Aging is a factor. That is, older alcoholics exhibit reduced capacity for recovery compared with younger alcoholics (Fein et al. 1990; Munro et al. 2000; Reed et al. 1992; Rourke and Grant 1999). Longer periods of abstinence may be required for follow-up investigations. Some brain damage, such as neuronal loss (Harper 2007), may be irreversible, even with extended abstinence.

Despite evidence for recovery of brain volume with abstinence, the mechanisms accounting for recovery remain unclear. One hypothesis, brain rehydration, was not supported by early human research studies (Schroth et al. 1988). An alternative explanation suggests that new neurons are created (i.e., neurogenesis) (e.g., Mandyam and Koob 2012): It is unlikely, however, that enough neurons could be made to replace the volume loss observed in chronic alcoholism. Nor is it clear that new neurons can migrate from neurogenic zones to distant areas of volume loss (Rakic 2002). On the other hand, adequate volume recovery may be explained by white-matter regeneration, because glial cells (i.e., oligodendrocytes) have the capacity to repair myelin and remyelinate neurons (Kipp et al. 2012), and oligodendrocyte progenitor cells have the potential to migrate long distances (Tirotta et al. 2010). Indeed, alcoholics who relapse have decreased white matter (Pfefferbaum et al. 1995), whereas continued abstinence is associated with increased white matter (Shear et al. 1994), notably in the corpus callosum and subcortical white matter (Cardenas et al. 2007).

Turning from studies with humans to animals, the following section examines imaging studies in models of alcoholism and related disorders.

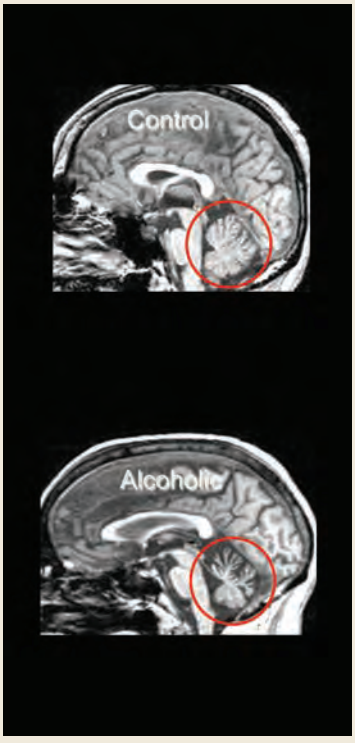

Figure 6 Cerebellar volume deficits in uncomplicated alcoholism. Midsagittal view of the brain, showing smaller volume of the anterior superior vermis of the cerebellum in an alcoholic man (bottom) compared with an age-matched control man (top).

Using Animal Models and Structural MRI to Study Alcoholism-Related Brain Disease

WE

There are two experimental approaches to model WE in rodents. The slower approach uses a thiamine-deficient diet (i.e., feeding with a thiamine-deficient chow), which can take 3–4 weeks to produce symptoms. Behavioral symptoms can be achieved in ~2 weeks using a combination of a thiamine-deficient chow and intraperitoneal (i.p.) administration of a thiamine pyrophosphokinase inhibitor such as pyrithiamine (Hazell and Butterworth 2009). Both models result in symptoms that mimic those observed in humans with WE (Pitkin and Savage 2001). Structural MRI findings in thiamine-deficient animals show similar patterns of brain changes, including hyperintense signals observed on T2-weighted images in thalamus, collicular bodies (Dror et al. 2010; Jordan et al. 1998; Pfefferbaum et al. 2007; Zahr et al. 2014*a*), hypothalamus, hippocampus (Jordan et al. 1998), mammillary bodies (Pfefferbaum et al. 2007), corpus callosum, and superior cerebellar peduncles (Dror et al. 2010). Thiamine deficiency may cause degeneration through neuroinflammatory mechanisms (Abbott 2000; Hazell and Butterworth 2009). In rats, inflammatory genes were highly expressed in vulnerable brain regions (Vemuganti et al. 2006). MRI in animal models permits further probing of the effects of thiamine deficiency on the brain and can be used to determine susceptible brain regions as a function of time of insult (Dror et al. 2010; Zahr et al. 2014*a*) as well as relationships between neuroinflammatory markers and brain insult (Zahr et al. 2014*a*). Such studies have also been used to confirm a mechanism of toxicity suspected based on research in humans (e.g., Harper 1980; Koguchi et al. 2004; Navarro et al. 2008): that glucose loading in a thiamine-deficient state can precipitate WE (Jordan et al. 1998; Zahr et al. 2014*a*), likely involving a breakdown of the blood–brain barrier (Nixon et al. 2008; Zelaya et al. 1995). In animals, postmortem followup can be used to confirm and extend in vivo findings. For example, electron microscopy showed a higher percentage of small fibers and myelin thinning in the corpus callosa of thiamine-deficient animals relative to controls (He et al. 2007).

Research with animals demonstrates that thiamine deficiency impairs several biochemical pathways requiring the thiamine derivative thiamine pyrophosphate (e.g., transketolase, pyruvate dehydrogenase, and α-ketoacid dehydrogenase) (Thomson et al. 2012), thereby interfering with carbohydrate metabolism (for energy production), lipid metabolism (for production and maintenance of myelin), and amino acid metabolism (for production of glucose-derived neurotransmitters; for example, glutamate and γ-aminobutyric acid [GABA]) (Sechi and Serra 2007; Vetreno et al. 2012). Consequently, the function of essential thiamine-requiring enzymes in the brain (e.g., transketolase, pyruvate dehydrogenase, and α-ketoacid dehydrogenase) is compromised, leading to oxidative stress, cellular energy impairment, and eventually neuronal loss (Thomson et al. 2012).

Evidence also shows that thiamine deficiency alters norepinephrine, dopamine (Mousseau et al. 1996), serotonin (Nakagawasai et al. 2007), and histamine (Langlais et al. 2002; McRee et al. 2000) synthesis and catabolism pathways. Thiamine deficiency may target focal brain areas such as the thalamus because, relative to other brain structures, it has lower levels of monocarboxylic acid transporters and acetyl-CoA-synthetase. This makes these areas less capable of generating energy from acetate (Qin and Crews 2014), which is a potential source of cellular energy in place of glucose in alcoholism (Volkow et al. 2013).

Current rodent models to study HE include models of acute and chronic liver failure (Butterworth et al. 2009; Diaz-Gomez et al. 2011). According to the International Society for Hepatic Encephalopathy, however, "At this time, there are no satisfactory animal models of Type C HE resulting from end-stage alcoholic liver disease or viral hepatitis, the most common etiologies encountered in patients" (Butterworth et al. 2009, p. 783). In addition, no MR-imaging studies to date have used rodent models of HE. Imaging studies in cats, dogs, and monkeys (Moon et al. 2012; Torisu et al. 2005; Zhou et al. 2012) typically recapitulate the human condition, showing nonspecific sulcal widening and hyperintensities in lentiform nuclei (i.e., putamen and globus pallidus of the basal ganglia) (Torisu et al. 2005; Zhou et al. 2012). Animal models of HE have been used to evaluate potential mechanisms of pathology, such as the contribution of excess ammonia in the blood (i.e., hyperammonemia) (Cauli et al. 2014) or lactate (Bosoi et al. 2014). Animal models of HE have also been used to explore treatment strategies for HE (e.g., hypothermia) (Barba et al. 2008).

CPM

Using a rat model of CPM to study white-matter degeneration, it was found that blood–brain barrier breakdown, detected with MRI, was associated with a higher risk of developing demyelination, as detected using postmortem histopathology (Adler et al. 2000). This study demonstrated that blood–brain barrier disruption exposes oligodendrocytes to substances normally excluded from the brain. This supports hypotheses from human postmortem studies suggesting that damage to the pons may be linked to reduced blood flow, as indicated by findings that basilar artery architecture is altered in CPM (De Reuck et al. 1975).

Although there are no known studies using structural MRI in animal models of ACD, ARD, or MBD, the following secti

Structural MRI Findings in Animal Models of Uncomplicated Alcoholism

An important initial report in the rodent MRI literature was the demonstration that brain growth continues beyond what would be considered adulthood in rats bred to prefer alcohol (i.e., alcohol-preferring rats, or P rats). Indeed, whole-brain volume in such rats continued to grow until approximately postnatal day 450 (Sullivan et al. 2006*a*), well past adulthood, which is typically considered as postnatal day 90 (Bell et al. 2013). Baseline studies (in the absence of alcohol [i.e., EtOH] exposure) also suggest that brains of alcohol-preferring rats are different relative to their wild-type counterparts, including reduced gray-matter volume in thalamus, ventral tegmental area, and insular and cingulate cortices (Gozzi et al. 2013).

One of the most consistent findings in alcohol-exposed rodents, ventricular enlargement, varies with timing and method of alcohol exposure. It is far more pronounced in rats achieving average blood alcohol levels (BALs) of 250 mg/dL in just 4 days of involuntary binge-type administration of EtOH (Zahr et al. 2010, 2013, 2014*b*) than in rats achieving average BALs of 200 mg/dL over 24 weeks using vapor EtOH exposure (Pfefferbaum et al. 2008) or P rats gradually achieving average BALs of 125 mg/dL with voluntary EtOH consumption (Pfefferbaum et al. 2006*a*), where only modest ventricular enlargement was noted (cf., Fadda and Rossetti 1998; Nixon 2006). Even repeated binge exposures (i.e., 5 cycles of 4 days of intragastric binge EtOH exposure with 1 week abstinence in between), do not result in persistent effects on the brain detectable with MRI (Zahr et al. 2015). Although ventricular size increases with each binge EtOH exposure, there is rapid recovery during each week of abstinence (Zahr et al. 2015). Such studies suggest that EtOH alone, at least in the exposure protocols evaluated with MRI, does not result in the characteristics observed in human alcoholics. Conversely, rats exposed to vaporized EtOH during adolescence are reported to show persistent effects (i.e., ventricular enlargement and deficits in hippocampal volume) into adulthood (Ehlers et al. 2013; Gass et al. 2014). Mice exposed to EtOH during adolescence are similarly purported to exhibit long-lasting regional brain-volume deficits in the olfactory bulb and basal forebrain (Coleman et al. 2011, 2014). These results suggest that the adolescent rodent brain may be more vulnerable to enduring toxic effects of EtOH than the adult rodent brain.

In monkeys trained to voluntarily consume alcohol, those that drank at least 3 g/kg EtOH per day for 15 months showed significant brain-volume shrinkage in the cerebral cortices (Kroenke et al. 2014). Because these animals were well nourished, these results suggest a direct relationship between oral EtOH intake and measures of decreased brain gray-matter volume.

Microstructural DTI

A number of sources provide extensive descriptions of the principles of DTI (Basser and Jones 2002; Chien et al. 1990; Gerig et al. 2005; Jones 2005; LeBihan 2001, 2003; Pierpaoli et al. 1996; Poupon et al. 1999; Sullivan and Pfefferbaum 2011). Briefly, DTI takes advantage of the fact that MR images of the brain are predominantly maps of water protons with contrast created by their immediate environment and their motility. In regions with few or no constraints imposed by physical boundaries, such as CSF in the ventricles, water movement is random and uniform in every direction and is therefore isotropic. In contrast to CSF, the path of a water molecule along a white-matter fiber is constrained by physical boundaries such as the axon sheath, causing greater movement along the long axis of the fiber than across it. This movement is called anisotropic; diffusion along the long axis of a fiber (axial or longitudinal diffusion) is greater than diffusion across the fiber (radial or transverse diffusion) (Song et al. 2002).

DTI findings are described in terms of diffusion. The magnitude of diffusion, referred to as mean diffusivity (MD) or the apparent diffusion coefficient (ADC), is calculated mathematically. Increased MD corresponds to white-matter damage. Fractional anisotropy (FA), ranging between 0 and 1, reflects axonal integrity, with lower integrity reflected by FA values closer to 0. Thus, disruption of white-matter microstructure detectable with DTI can reflect compromised myelin, cytoskeletal structure, or axonal density (Basser 1995; Basser and Pierpaoli 1996; Spielman et al. 1996).

Several approaches have been used to quantify DTI metrics. One of the more desirable approaches is the use of quantitative fiber tracking, which is able to evaluate fibers along their entire length and can thus detect compromised white matter. This technique can be used to depict selective commissures (e.g., corpus callosum), projection fibers, and association fibers.

DTI Findings in Alcoholism-Related Brain Disorders

In one study, DTI in alcoholics with (n = 7) and without (n = 20) WKS showed FA deficits in the fornix and cingulum bundle of the Papez or medial limbic circuit, measured using tract-based spatial statistics (TBSS). These FA effects were greater in alcoholics with WKS relative to those without it (Segobin et al. 2015). The number of tracts in the fornix appears to be reduced only in WKS patients (Nahum et al. 2015).

Studies of people who have alcohol-related cirrhosis with HE have reported elevated MD in several white-matter bundles, including the corpus callosum, internal capsule, and frontal white matter (Kale et al. 2006), and effects on both FA and MD of occipital white matter (Kumar et al. 2008). HE caused by alcoholism compared

with other forms of HE (e.g., as a result of viral infection or primary biliary cirrhosis) appears to have different effects on DTI parameters (Miese et al. 2006), with more widespread changes in FA and MD in alcoholic relative to nonalcoholic cirrhosis (Ahluwalia et al. 2015). When researchers induced hyperammonemia in cirrhotic patients, an increase in ADC in brain white matter was observed, supporting excess ammonia in the blood as a mechanism driving cerebral edema (Mardini et al. 2011).

DTI showed elevated MD in the middle cerebellar peduncles with no effects on corticospinal tracts in a study participant with CPM relative to three healthy comparison participants (Min et al. 2012; Nair et al. 2012).

The largest DTI study of MBD to date included six study participants, five with a history of chronic alcoholism. All six showed hyperintense signals on diffusion images and low ADC of the corpus callosum. Researchers observed cortical lesions in frontoparietal regions in three of six study participants with the poorest outcomes (Menegon et al. 2005). Remaining DTI studies of MBD were case studies (e.g., Tuntiyatorn and Laothamatas 2008) showing low ADC along the entire corpus callosum (Bano et al. 2009; Wenz et al. 2014), with FA values diminishing progressively from front to back (Pacheco et al. 2014; Sair et al. 2006). No known DTI studies have been conducted in patients with ACD or ARD.

DTI Findings in Uncomplicated Alcoholism

DTI has revealed microstructural damage related to alcoholism in cerebral areas that appear intact in structural MRI analyses (e.g., Pfefferbaum and Sullivan 2002; Pfefferbaum et al. 2006*b*; Sullivan et al. 2003). Corpus callosum findings in uncomplicated alcoholics are common and, as observed for MBD, show greater anterior than posterior effects (e.g., Arnone et al. 2006; Konrad et al. 2012; Liu et al. 2010; Pitel et al. 2010; Schulte et al. 2005). Quantitative fiber tracking has demonstrated greater FA deficits in anterior than in posterior fibers of supratentorial and infratentorial white-matter bundles in alcoholics compared with healthy controls, as well as low FA in tracts of the corpus callosum, centrum semiovale, internal and external capsules, fornix, superior cingulate, and longitudinal fasciculi (Fortier et al. 2014; Müller-Oehring et al. 2009; Pfefferbaum and Sullivan 2005; Pfefferbaum et al. 2000, 2002*a*, 2009*a*; Trivedi et al. 2013). Fronto-limbic (Harris et al. 2008; Monnig et al. 2013), fronto-parietal (Maksimovskiy et al. 2014), fronto-occipital (Bagga et al. 2014), fronto-cerebellar (Sullivan and Pfefferbaum 2005), cortico-striatal (Yeh et al. 2009), and cortico-pontine (Chanraud et al. 2009*b*) fibers are also affected in alcoholics relative to healthy controls.

Studies have also examined DTI-function relationships in alcoholism. FA in anterior cingulate and motor areas correlates with executive and psychomotor performance (Konrad et al. 2012), FA in the splenium correlates with working memory (Pfefferbaum et al. 2000), and FA in several regions (corpus callosum, parietal, occipital, and frontal white-matter) correlates with performance on the Iowa Gambling Task (Zorlu et al. 2013). A double dissociation was found showing that higher diffusivity in sensory-motor and parietal bundles was associated with poorer balance but not psychomotor speed, whereas higher diffusivity in prefrontal and temporal bundles was associated with slower psychomotor speed but not balance (Pfefferbaum et al. 2010). DTI changes in multiple supratentorial and infratentorial fiber systems in alcoholics correlated with impairment in speeded performance and postural stability (Pfefferbaum et al. 2009*b*), frontal fiber integrity connecting left and right hemispheres predicted performance on a coordinated psychomotor task (Rosenbloom et al. 2008), and number of reconstructed fibers running between the pons and the midbrain was related to cognitive flexibility performance (Chanraud et al. 2009*b*). Gray-matter diffusivity in the hippocampus, which is lower in alcoholics than in healthy controls, is related to episodic memory impairment (Chanraud et al. 2009*a*).

DTI Findings in Recovery from Alcoholism

Similar to structural MRI findings demonstrating pronounced tissue-volume shrinkage of orbitofrontal cortices in abstinent alcoholics who were likely to resume drinking (e.g., Beck et al. 2012; Cardenas et al. 2011; Durazzo et al. 2011), DTI identified alcoholic individuals more likely to resume drinking 6 months following initial evaluation based on lower FA and higher diffusivity in frontal white matter at baseline (Sorg et al. 2012). Increases in FA and decreases in diffusivity have been interpreted as evidence for white-matter recovery with abstinence. Studies have shown recovery in corpus callosum at 1 year compared with 2 weeks of abstinence (Alhassoon et al. 2012) and in frontal white matter at 1 month compared with 1 week of abstinence, at least in nonsmoking, sober alcoholics (Gazdzinski et al. 2010). Other reports suggest that some white-matter impairments persist after 6 to 30 months of recovery in alcoholics relative to healthy controls (Zorlu et al. 2014). In a seminal longitudinal study of 47 alcoholic and 56 healthy controls study participants, Pfefferbaum and colleagues (2014) reported that, despite abnormally low FA, age trajectories of the alcoholics who abstained were positive and progressing toward normality, whereas those of the relapsing alcoholics and control subjects were negative.

DTI Findings in Animal Models of WE

DTI data have been collected in animal models of WE but not in other concomitants of alcoholism.

In the study in which WE was induced by thiamine deficiency, animals were imaged at baseline, presymptomatic stage (day 10), symptomatic stage (days 12 and 14), and after recovery on days 31 and 87. A decrease in FA in the inferior colliculi was first noted on day 10 but showed recovery on day 87. On the other hand, the FA decrease in the thalamus first noted on day 12 persisted through day 87 (Dror et al. 2010). This model was also used in a pharmacological DTI study in which animals were exposed to rasagiline, a selective monamine oxidase B inhibitor, as a potential protective agent against thiamine-deficiency–induced brain damage (Dror et al. 2014). In addition to reducing ventricular enlargement, rasagiline appeared to ameliorate the effects of thiamine deficiency on the FA decrease in the thalamus (Dror et al. 2014). Histopathology showed that treatment with rasagiline reduced the lesions in thalamus and colliculi observed in the thiamine-deficient brain (Eliash et al. 2009). Rasagiline has not been evaluated in human patients with WE.

DTI Findings in Animal Models of Uncomplicated Alcoholism

Adolescent animals exposed to intermittent EtOH and evaluated postmortem showed no effects on FA but reduced axial diffusivity (hippocampus, cortex, and cerebellum), reduced radial diffusivity (hippocampus and cortex), and reduced MD (cerebellum and corpus callosum) in several brain regions (Vetreno et al. 2016). Adult rats exposed to a single dose of EtOH showed a slight and transient reduction, relative to unaffected rats, in ADC in brainstem (Kong et al. 2013), frontal lobe, hippocampus, thalamus, and cerebellum (Liu et al. 2014). These findings were interpreted as reflecting the development of cytotoxic brain edema, as histological analysis showed cell swelling and narrowed extracellular spacing (Kong et al. 2013).

Whereas chronic exposure to vaporized EtOH did not result in detectable effects on FA or MD, binge EtOH exposure resulted in transient decreases in FA and transient increases in MD (Pfefferbaum et al. 2015). Together, these results suggest that DTI can detect acute and subchronic effects on the brain, but that chronic exposure to EtOH can result in brain adaptations such that effects on FA and MD are no longer discernable.

Magnetic Resonance Spectroscopy

Although MRI primarily depicts the distribution of water protons, similar technology can also be used to obtain information about chemical constituents other than water, primarily due to a small frequency shift, or "chemical shift," relative to the water signal. The acquisition of MR-detectable signals other than those of water and fat is referred to as MRS and is an in vivo application of traditional laboratory-based NMR spectroscopy.

MRS reveals information about several biochemicals, or metabolites, in the brain. The largest signals arise from *N*-acetylaspartate (NAA), creatine and phosphocreatine (i.e., total creatine [tCr]), and choline-containing compounds (Cho). Signals from the combined resonances of glutamate (Glu) and glutamine (Gln) (i.e., Glx) are also sometimes reported, as are myo-inositiol (mI) and lactate (lac). Signals from Glu and GABA can also be detected under certain conditions.

MRI and Signals for Four Prominent Metabolites

NAA

The predominant in vivo proton signal is NAA, with contributions from other *N*-acetyl compounds, especially *N*-acetyl aspartyl glutamate. NAA is found almost exclusively in neurons (Petroff et al. 1995; Urenjak et al. 1992, 1993) and, thus, is considered a measure of neuronal integrity. Postmortem (Cooper 1972; Koller et al. 1984; Nadler and Cooper 1972) and MRS (Kwo-On-Yuen et al. 1994; Petroff et al. 1995) studies have shown NAA levels to be higher in gray than in white matter in healthy study participants, as have in vivo studies (Doyle et al. 1995; Lim and Spielman 1997; Lim et al. 1998; Moyher et al. 1995; Narayana et al. 1989; Pouwels and Frahm 1998; Schuff et al. 1999; Wang et al. 1998).

tCr

The tCr signal, generated by creatine and phosphocreatine, is influenced by the state of high-energy phosphate metabolism (Tedeschi et al. 1995). In spectroscopy studies, it often is used as a reference for other peaks based on the incorrect assumption that its concentration is relatively constant (cf. Zahr et al. 2008, 2009, 2014*b*).

Cho

The in vivo MRS-visible Cho peak is generated primarily by water-soluble choline-containing compounds (free choline, phosphocholine, and glycerophosphocholine) (Barker et al. 1994) and is associated with cell-membrane synthesis and turnover. The Cho resonance also provides an index of cellular density in brain tumors (Gupta et al. 1999) and may be a marker of increases in glial density with age and disease. MRS-measured Cho concentration is higher in white than gray matter (Pfefferbaum et al. 1999) and increases with normal aging (Chang et al. 1996; Kreis et al. 1993; Moats et al. 1994; Pfefferbaum et al. 1999; Soher et al. 1996).

mI

Myo-inositiol is present in glial but not neuronal cell cultures (Brand et al. 1993; Petroff et al. 1995) and plays a role in maintaining cell volume (Ernst et al. 1997; Lien et al. 1990). The concentration of mI is higher in

gray than in white matter (Michaelis et al. 1993; Pouwels and Frahm 1998).

Figure 7 shows a graph of MR spectra from the thalamus of a 55-year-old nonalcoholic woman. The major metabolites are color coded.

MRS Findings in Alcoholism-Related Brain Disorders

As with the other imaging modalities, MRS reports of WE are primarily case studies. For example, a Japanese man who had consumed alcohol for 50 years and had eaten poorly for several days as a result of a cold presented with gait disturbances and incoherent speech. MRS before and after thiamine treatment found an initial low level of NAA/tCr in the thalamus, which appeared to increase with thiamine replacement. NAA/tCr levels in the cerebellum did not increase, although a lactate peak initially present in the cerebellum resolved (Murata et al. 2001). MRS conducted in two patients with non–alcohol-related thiamine deficiency (i.e., caused by gastric and pancreatic cancer) compared with five healthy study participants showed similar results: relatively low NAA/tCr levels in the thalamus resolved after treatment with thiamine (Mascalchi et al. 2002).

In a variety of brain regions, MRS findings in alcohol-related cirrhosis and HE are remarkably consistent and comparable with findings in nonalcoholic HE (e.g., Cordoba et al. 2001; Gupta et al. 1993; Häussinger et al. 1994), showing lower levels of Cho/tCr and mI/tCr and higher levels of Gln/tCr (Ahluwalia et al. 2015; Binesh et al. 2006; Chavarria et al. 2013; Jain et al. 2013; Kreis et al. 1992; Laubenberger et al. 1997; Miese et al. 2006; Pujol et al. 1996; Singhal et al. 2010; Taylor-Robinson et al. 1994, 1999; Thomas et al. 1998). Levels of mI and Cho are lowest and Glx highest in patients with HE (Geissler et al. 1997; Lee et al. 1999; Poveda et al. 2010; Ross et al. 1994; Tarasow et al. 2003).

Mild swelling of astrocytes is proposed as the key event in the pathogenesis of HE (e.g., Takahashi et al. 1991). In cirrhosis, elevated blood level of ammonia is thought to result in elevated brain ammonia, which can be toxic (Weissenborn et al. 2007). It often has been proposed that the brain's response to elevated ammonia levels is to combine ammonia and glutamate to make glutamine using glutamine synthetase, found primarily in astrocytes (Yamamoto et al. 1987). Thus, brain swelling in cirrhosis is thought to reflect an increase in astrocytic glutamine formation. The decrease in mI is thought to be a compensatory mechanism to counterbalance the osmotic effect of cerebral glutamine accumulation (Balata et al. 2003; Mardini et al. 2011). Although some articles claim to measure in vivo glutamine (e.g., Binesh et al. 2006; Chavarria et al. 2013; Jain et al. 2013; Kreis et al. 1992; McConnell et al. 1995), it is unlikely that the MRS method used in these cases permitted the separate detection of glutamate and glutamine, which are strongly coupled and difficult to detect independently, even with very short echo times (Adalsteinsson et al. 2002). A single study measured GABA levels in five alcoholics without HE and five study participants with both alcohol and non–alcohol-related HE. GABA levels were lower in the two patient groups relative to 10 comparison participants (Behar et al. 1999).

In the only report of MRS conducted in a case of alcoholism-associated CPM, elevated Cho/tCr was found and interpreted as reflecting edema or demyelination in a 53-year-old man with gait disturbances and hearing loss (Nomoto et al. 2004). In 12 patients with chronic hyponatremia (nonalcohol etiology), MRS showed reduced Cho and mI relative to unaffected study participants, reflecting osmolyte disturbances (Videen et al. 1995).

Research also has found compromised NAA/tCr levels in patients with cerebellar degeneration (Tedeschi et al. 1996; Terakawa et al. 1999). Two MRS case studies of MBD showed reduced NAA/tCr and elevated Cho/tCr in corpus callosum splenium (Gambini et al. 2003;

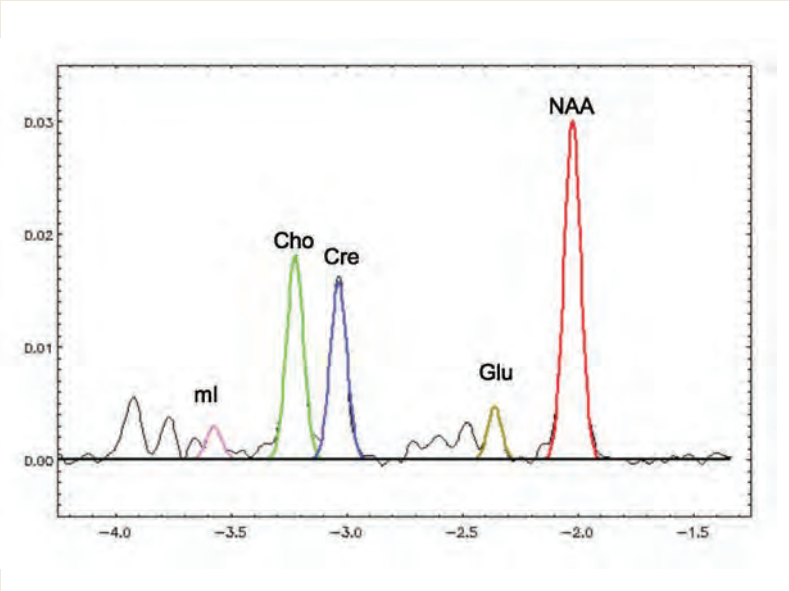

Figure 7 Magnetic resonance spectroscopy spectra from the thalamus of a 55-year-old nonalcoholic control woman, with a gaussian fit of the major metabolites that has been color coded.

Tuntiyatorn and Laothamatas 2008), findings consistent with demyelination (elevated Cho) and axonal injury (reduced NAA).

MRS Findings in Uncomplicated Alcoholism

Most MRS studies show lower levels of NAA in recently sober alcoholics relative to healthy controls in several brain regions, including frontal areas (Bendszus et al. 2001; Durazzo et al. 2004, 2010; Fein et al. 1994; Jagannathan et al. 1996; Meyerhoff et al. 2004; Schweinsburg et al. 2003; Seitz et al. 1999) and cerebellum (Bendszus et al. 2001; Durazzo et al. 2010; Jagannathan et al. 1996; Parks et al. 2002; Seitz et al. 1999). Similarly, studies in AUD patients shortly following detoxification have found low levels of Cho (Bendszus et al. 2001; Durazzo et al. 2004; Ende et al. 2005; Fein et al. 1994; Parks et al. 2002; Seitz et al. 1999), although Cho findings in AUD are less consistent (e.g., Hermann et al. 2012; Modi et al. 2011). Because these findings are prominent in white matter, it is thought that the effects of alcoholism are greater in white than in gray matter (De la Monte 1988; Harper et al. 2003).

MRS Findings in Recovery from Alcoholism

MRS studies suggest that NAA (e.g., Bartsch et al. 2007; Bendszus et al. 2001; Parks et al. 2002), particularly in frontal (Bartsch et al. 2007; Bendszus et al. 2001; Durazzo et al. 2006) and cerebellar (Bendszus et al. 2001; Fein et al. 1994; Parks et al. 2002) regions and Cho levels (e.g., Bartsch et al. 2007; Bendszus et al. 2001; Durazzo et al. 2006; Ende et al. 2005; Martin et al. 1995) show normalization (i.e., increase) with abstinence. Elevations in mI are not seen in long-term sober alcoholics (Schweinsburg et al. 2000). These findings suggest that low NAA levels initially observed in recently sober alcoholics reflect neurodegeneration without cell death, and increases with abstinence may reflect healing without cell generation. The disruption and recovery of Cho and mI levels suggest white-matter recovery with sobriety and the potential for remyelination.

MRS Findings in Animal Models of Syndromes Associated With Alcoholism

In rat models of WE induced using pyrithiamine, the dominant MRS pattern is a reduction in both NAA and Cho in several brain regions (Lee et al. 1995, 2001; Rose et al. 1993), including the thalamus (Navarro et al. 2008, 2005; Pfefferbaum et al. 2007). Researchers also frequently report elevations in lactate (Navarro et al. 2005, 2008). Precipitation of WE with glucose (resulting in seizures) is associated with further decreases in NAA and Cho and, significantly, an elevation in lactate (Zahr et al. 2014*a*). Treatment with thiamine is associated with recovery in Cho levels (Lee et al. 1995).

MRS has been used to evaluate models of HE achieved using various methods (Cudalbu 2013) and most reports show similar findings. Hepatic devascularization (Barba et al. 2008; Zwingmann et al. 2004), carbon tetrachloride treatment (Bates et al. 1989), bile-duct ligations (Bosoi et al. 2014; Rackayova et al. 2015), and other means of promoting hyperammonemia (e.g., acute liver ischemia, urease, or methionine sulfoximine treatment) (Bosman et al. 1990; de Graaf et al. 1991) result in elevated levels of Gln and frequently lactate in rat brain (e.g., cortex). Additional effects reported include lower levels of NAA, mI, Cho, and Glu (Barba et al. 2008; Bates et al. 1989; Bosman et al. 1990; de Graaf et al. 1991; Peeling et al. 1993; Rackayova et al. 2015; Zwingmann et al. 2004). As in the human condition, a similar caveat holds: that is, it is not clear if Glu and Gln are clearly discriminated in many of these studies, and, often, reports more likely reflect Glx levels.

Although in vivo MRS studies in both humans and animals have persisted in interpreting elevations in brain Gln as reflecting elevations in peripheral ammonia and brain edema (Venkatasubramanian et al. 2001), ex vivo carbon 13 nuclear MR studies have challenged the convention that glutamine accumulation is the major cause of brain edema in acute HE. Such studies instead indicate limited metabolic pathway reactions and capacity of astrocytes to detoxify ammonia by glutamine synthesis and emphasize distortions of energy and neurotransmitter metabolism (Zwingmann 2007).

MRS Findings in Animal Models of Uncomplicated Alcoholism (and Recovery)

MRS can be used in animals to detect and quantify in vivo and real-time brain EtOH kinetics (e.g., rats: Sullivan et al. 2005*c*; monkeys: Kaufman et al. 1994). Unlike findings in long-term sober human alcoholics, nonabstinent chronic heavy drinkers (Meyerhoff et al. 2004) and social and moderate drinkers (Ende et al. 2006) show elevated levels of brain Cho. Elevated levels of Cho are also reported in the thalamus of rodents between weeks 16 and 40 of alcohol exposure (Lee et al. 2003). Neuroimaging research has been conducted with rodent models of binge (Zahr et al. 2010, 2013, 2014*b*), repeated binge (Zahr et al. 2015), and chronic alcohol exposure (Zahr et al. 2009). In vivo MRS studies have consistently shown that a single 4-day binge exposure with BALs approaching 300 mg/dL is associated with reversible changes to the brain: levels of NAA are lower and those of Cho are higher following binge EtOH exposure (Zahr et al. 2010, 2013, 2014*b*). In the repeated-binge experiment, animals were exposed to 5 cycles of 4 days of intragastric EtOH treatment and 10 days of recovery. Changes in MRS metabolite levels again were transient: levels of NAA decreased, whereas those of Cho increased with each

binge EtOH exposure cycle but then recovered during each abstinence period. Changes in response to EtOH were in expected directions based on the previous, single-binge EtOH exposure experiments but did not accrue with repeated-binge EtOH exposure (Zahr et al. 2015). In the chronic EtOH exposure study, NAA levels were lower in the EtOH-exposed relative to the comparison group but did not attain statistical significance, whereas levels of Cho appeared to demonstrate a dose-response curve (i.e., increasing levels with higher and longer EtOH exposure) (Zahr et al. 2009).

Conclusion

Imaging investigations of alcohol-related brain disorders show unique neuropathology (as outlined in table 1), offering a framework for examining pathology in uncomplicated alcoholism. Because brains affected by AUD can show mild effects in the regions aggressively targeted by overt disease, animal models have been useful in distinguishing the etiology of pathology and differentiating brain regions specifically targeted by thiamine deficiency versus hyperammonemia, for example. Individuals with AUD may show more prominent effects in some regions compared with others, suggesting a propensity for one diagnosis over another (e.g., an alcoholic may be more vulnerable to thiamine deficiency than to liver damage). What remains unresolved, and what animal models can help determine, is why certain brain regions are differentially vulnerable to certain pathologies. For example, are the colliculi sensitive to thiamine deficiency because of their relatively high metabolic rate (Landau et al. 1955; Sokoloff et al. 1977)? Is the pons susceptible to CPM because of its proximity to the basilar artery? Does dopamine explain why basal ganglia are targets of liver disease (Mousseau et al. 1993)?

In vivo imaging studies in humans and animal models will continue to provide an evolving picture of the course of alcoholic brain disease through remissions and exacerbations as long-term studies follow human alcoholics as they age and as new initiatives evaluate adolescents before they are exposed to alcohol.

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Neuroimmune Function and the Consequences of Alcohol Exposure

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Induction of neuroimmune genes by binge drinking increases neuronal excitability and oxidative stress, contributing to the neurobiology of alcohol dependence and causing neurodegeneration. Ethanol exposure activates signaling pathways featuring high-mobility group box 1 and Toll-like receptor 4 (TLR4), resulting in induction of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells, which regulates expression of several cytokine genes involved in innate immunity, and its target genes. This leads to persistent neuroimmune responses to ethanol that stimulate TLRs and/or certain glutamate receptors (i.e., N-methyl-D-aspartate receptors). Alcohol also alters stress responses, causing elevation of peripheral cytokines, which further sensitize neuroimmune responses to ethanol. Neuroimmune signaling and glutamate excitotoxicity are linked to alcoholic neurodegeneration. Models of alcohol abuse have identified significant frontal cortical degeneration and loss of hippocampal neurogenesis, consistent with neuroimmune activation pathology contributing to these alcohol-induced, long-lasting changes in the brain. These alcohol-induced long-lasting increases in brain neuroimmune-gene expression also may contribute to the neurobiology of alcohol use disorder.

Key words: Alcohol use, abuse, and dependence; alcohol effects and consequences; alcohol exposure; binge drinking; immunity; neuroimmune responses; neuroimmune genes; neurodegeneration; brain; microglia; stress axis; stress responses; oxidative stress; glutamate receptors; Toll-like receptors; cytokines; high-mobility group box 1; nuclear factor-kappa B

To a large extent, signaling processes between neurons in the brain are distinct from signaling mechanisms between cells in the immune system and use different signaling molecules. However, some proteins first discovered within the immune system act as both peripheral immune-signaling molecules and brain-signaling molecules. These neuroimmune factors include various cytokines, Toll-like receptors (TLRs), and high-mobility group protein box 1 (HMGB1). In the brain, both neurons and supporting glial cells (both astrocytes and microglia) contribute to the release of and responses to these neuroimmune factors. Neuroimmune signaling in the brain not only is a part of the innate immune response, but its effects also persist for long periods and could contribute to long-lasting changes in neurobiology.

Studies found that brain neuroimmune signaling is activated in models of binge drinking and neurodegeneration, suggesting another pathway through which alcohol may affect brain function. This review defines the roles of various cellular compartments and signaling molecules involved in neuroimmune activation, including the role of the stress axis in the communication between the central and peripheral immune systems and in sensitizing the neuroimmune response to alcohol. The article also will offer evidence from animal studies and postmortem human alcoholic brain studies that neuroimmune signaling may increase alcohol drinking and risky decision making and (in alcohol-treated animals) blunt the ability to change, decreasing behavioral flexibility.

Neuroimmune Signaling in the Alcoholic Brain

Monocytes and Innate Immune Genes

Innate immune genes are associated with rapid first-line responses to infections that involve primarily immune cells called monocytes (e.g., the acute-

phase response). These responses include increases in multiple cytokines as well as in their cellular receptors. Together, these changes amplify expression of a large number of genes through kinase signaling pathways that converge on two transcription factors called nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein-1 (AP-1). NF-κB and AP-1 promote expression of innate immune cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), as well as of TLRs and cytokine receptors (see figure 1). In addition, innate immune responses include the activation of proteases and oxidases, particularly cyclooxygenase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,[1] as well as of major histocompatibility complex (MHC) signaling molecules, such as beta-2 microglobulin.

[1] NADPH oxidase is an enzyme that produces reactive oxygen species (ROS)—for example, during ethanol metabolism—thereby increasing oxidative stress and contributing to cell damage.

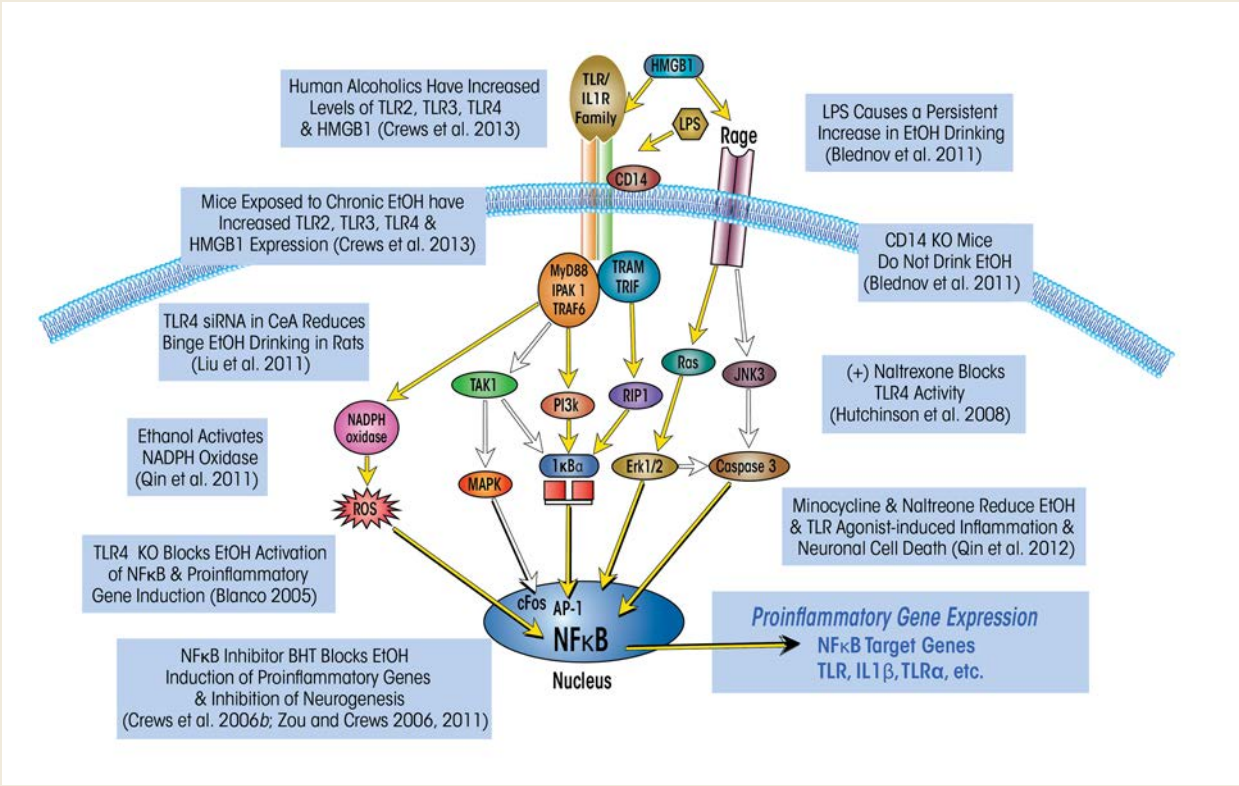

Figure 1 Simplified schematic of the Toll-like receptor (TLR) and the receptor for advanced glycation end products (RAGE) signaling cascades. Stimulation of TLRs with high-mobility group box 1 protein (HMGB1) and other inflammation-inducing agents leads to the generation of reactive oxygen species (ROS) and downstream activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF)-κB. Similarly, HMGB1 activation of the RAGE receptor results in downstream activation of NF-κB and induction of ROS. Transfer of NF-κB to the nucleus induces proinflammatory gene expression, neuroimmune induction, and cell death. Expression of several TLRs (i.e., TLR2, TLR3, and TLR4) and HMGB1 is upregulated in the postmortem human alcoholic brain and mouse brain following exposure to ethanol (Crews et al. 2013); this is accompanied by an upregulation of NADPH oxidase expression (Qin et al. 2011). Interestingly, blockade of neuroimmune signaling, either genetically (Blanco 2005) or pharmacologically (Crews et al. 2006*b*; Qin et al. 2012; Zou and Crews 2006, 2011), prevents ethanol-induced neuroimmune-gene induction and neurodegeneration. The neuroimmune system also contributes to alcohol-drinking behavior, because activation (Blednov et al. 2001) or blockade of this system (Blednov et al. 2011; Liu et al. 2011) increases and decreases self-administration, respectively.

NOTE: AP-1: activator protein-1; CD14: cluster of differentiation 14; ERK: extracellular signal–regulated kinase; IKK: inhibitor of NF-κB; IRAK 1: interleukin-1 receptor–associated kinase 1; JNK: c-jun N-terminal kinases; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; MyD88: myeloid differentiation primary response gene 88; NADPH oxidase: nicotinamide adenine dinucleotide phosphate-oxidase; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; RIP: receptor interacting protein; TAK1: transforming growth factor beta–activated kinase 1; TRAF: tumor necrosis factor receptor–associated factor; TRAM: TRIF-related adaptor molecule; TRIF: TIR-domain-containing adaptor–inducing interferon-beta.
SOURCE: Adapted from Crews et al. 2011, 2013.

The NF-κB–mediated transcription of proinflammatory genes, in turn, is amplified within and across cells by induction of TLRs and cytokine receptors (e.g., those that belong to the IL-1β receptor family), which induce innate immune gene expression. Amplification of innate immune gene induction across cells and tissues can cause pathology, such as sepsis. Sepsis and systemic inflammatory-response syndrome involve a "cytokine storm." This potentially fatal innate immune reaction consists of positive feedback loops between cytokines and immune and tissue cells, resulting in highly elevated levels of cytokines in the blood, multiorgan failure, and death (Osterholm 2005). Models of sepsis that involve activation of an acute phase-like response lead to increases in the levels of multiple cytokines in the blood that occur in two distinct phases. First, both TNF-α and IL-1β levels increase during the first several hours after infection but then subside. Subsequently, levels of HMGB1, a ubiquitously expressed, cytokine-like protein that can activate TLR4 and potentiate cytokine responses, increase about 16 hours after infection and remain elevated for several days (Wang et al. 2001). In mouse models, sepsis-induced death that occurs several days after infection is associated with HMGB1 and is prevented by treatment with antibodies blocking HMGB1. Survivors of sepsis show prolonged increases in serum HMGB1 and cognitive deficits that can be prevented with HMGB1-antibody treatment (Chavan et al. 2012). About half of the patients released from the hospital after surviving a cytokine storm–sepsis insult die within 5 years (Quartin et al. 1997). Thus, innate immune responses can be long lasting and can induce pathology long after they initially have been activated. However, although most studies support a central role for NF-κB–mediated transcription of proinflammatory cytokines, proteases, and oxidases in the innate immune response, both the precise mechanisms that regulate individual cell or cytokine activation and the contributions of tissues and cells in vivo to amplification of specific innate immune genes are poorly understood.

Monocytes are the primary cells mediating the innate immune response. They are found in all tissues, including the brain. Monocytes in the brain, which also are referred to as microglia, fall into two main categories: proinflammatory M1 monocytes/microglia and trophic M2 monocytes/microglia. M1 monocytes/microglia participate in the acute proinflammatory responses of the innate immune system; in addition, they also convey signals to the adaptive immune cells (i.e., T and B cells) through the MHC molecules they carry on their cell surface. These signals help create a persistent sensitization to pathogens (e.g., in the form of antibodies that mediate immunization). These proinflammatory effects occur in response to pathogens as well as tissue damage, cell death, and degeneration. Thus, M1 microglia and other monocyte-like cells consistently express multiple cytokine receptors and TLRs that, when activated, induce innate immune genes, such as proinflammatory cytokines, proteases, and oxidases, which help to break down, process, and remove damaged cells and tissue. In contrast, the M2 monocytes/microglia mediate a delayed response that initiates wound-healing trophic signaling and seem to be critical for healing. Both monocytes in general and brain microglia in particular can have both proinflammatory M1 and trophic M2 phenotypes (Colton 2009; Michelucci et al. 2009).

Although the M1 and M2 phenotypes are poorly understood, monocyte proinflammatory activation clearly is linked to NF-κB–mediated transcription of multiple innate immune genes. Activation of monocyte NF-κB by both pathogens and tissue damage involves TLR4 (see figure 1). This receptor responds to endotoxin released by certain bacteria (e.g., lipopolysaccharide [LPS]) as well as to HMGB1. Proinflammatory gene induction also is amplified by cytokine–receptor-activated release of HMGB1 that further contributes to innate immune gene induction.

The role of these innate immune-signaling molecules is well characterized within the immune system, but only recently these molecules have been discovered to also contribute to brain signaling. Thus, studies have indicated that MHC molecules contribute to brain development (Huh et al. 2000), to most neurodegenerative diseases (Gage 2002; Glass et al. 2010), and to alcohol and other drug dependence (Crews 2012). Neuroimmune signaling in the brain has not been extensively studied, and most knowledge on this subject is based on the assumption that monocyte responses elsewhere in the body reflect microglial and brain innate immune responses.

The Immune Response in the Brain

The immune system is not normally active in the healthy brain. Thus, the healthy normal brain does not contain antibodies and has only one type of immune cell, the microglia. During fetal development, neurons, astrocytes, and all other brain cells are formed from one embryonic structure (i.e., the ectoderm), whereas microglia migrate from another embryonic structure (i.e., the mesoderm) to the brain at a specific time (Ginhoux et al. 2010). In the healthy brain, the number of ramified or "resting" microglia equals that of neurons, and these cells contribute to the integration of sensory systems and overall survey of the brain milieu (Raivich 2005). Along with astrocytes, they modulate important metabolic, trophic, and synaptic functions in addition to responding to brain–damage-induced neuroimmune responses (Farina et al. 2007; Streit et al. 2004). Microglia respond to endogenous or exogenous insults with distinct morphological changes in shape (i.e., they develop "bushy" or "amoeboid-like" pheno-

types) as well as with marked alterations in gene expression, including proinflammatory innate immune-response genes (Graeber 2010). However, it sometimes is unclear whether microglia are responding to a brain insult or causing it through the release of proinflammatory cytokines. Microglia respond to and signal through both neuroimmune and neurotransmitter signals. For example, acetylcholine—an important neurotransmitter involved in multiple brain functions, including cognition—inhibits proinflammatory activation in both peripheral monocytes and brain microglia and has anti-inflammatory effects.

Some studies found an increase in the expression of the microglial marker Iba-1 in the brains of alcoholic individuals (see figure 2) (He and Crews 2008), suggesting that microglia contribute to the neurobiology of alcoholism. Microglia in postmortem human alcoholic brain and chronic alcohol-treated mouse and rat brain show increased MHC gene expression, but not the bushy or phagocytic activation profiles associated with marked brain damage. Chronic ethanol treatment also increases microglial TLR4 expression (Vetreno et al. 2013). Thus, microglia are the only immune cells in healthy brain and are integrated into the brain's responses to both neurotransmitters and neuroimmune signals. They also seem to contribute to chronic alcohol-induced responses.

Alcohol, Neuroimmune Signaling, and Neurodegeneration

Chronic binge-drinking models repeatedly found that ethanol exposure increases the expression of a variety of neuroimmune genes in the brain and that these alterations may persist over long periods (see figure 1). For example, one study found that chronic ethanol exposure induced the neuroimmune gene cyclooxygenase 2 (COX2) in multiple cortical and limbic brain regions long after physical signs of withdrawal had subsided (Knapp and Crews 1999). However, ethanol did not induce COX2 in transgenic mice lacking TLR4, suggesting that this process involves TLR4 (Alfonso-Loeches et al. 2010). Chronic alcohol exposure also altered the activity of NF-κB and another regulatory protein, cyclic AMP-responsive element binding protein (CREB). Specifically, ethanol treatment of HEC brain slice cultures increased NF-κB binding to DNA probes modeling gene promoter regions and decreased CREB binding to DNA probes modeling CREB-responsive gene promoter DNA (Zou and Crews 2006).

The CREB family of transcription factors is activated by phosphorylation; they promote neuronal survival, protecting neurons from excitotoxicity and apoptosis by regulating the transcription of pro-survival factors (Lonze and Ginty 2002; Mantamadiotis et al. 2002). Conversely, NF-κB is known widely for its ubiquitous roles in inflammatory and immune responses (O'Neill and Kaltschmidt 1997). Accordingly, NF-κB and CREB have different target genes. For example, CREB targets the neuropeptide Y and brain-derived neurotrophic factor (BDNF) genes, both of which are involved in promoting neuronal growth and resilience to insults, including protection against excitotoxicity and neuronal death (Lonze and Ginty 2002). Regular excitation of neurons increases synaptic plasticity related to CREB and induces synaptic proteins and BDNF. In contrast, excessive excitation triggers activation of certain extrasynaptic receptors for the neurotransmitter glutamate (i.e., *N*-methyl-D-aspartate [NMDA] receptors) and excitotoxicity, resulting in either rapid or delayed neuronal death, which is associated with reduced CREB (Hardingham and Bading 2010). Chronic ethanol exposure interferes with the normal functions of CREB. Thus, the levels of CREB phosphorylation and CREB–DNA binding as well as of the target gene BDNF all were decreased in the rat

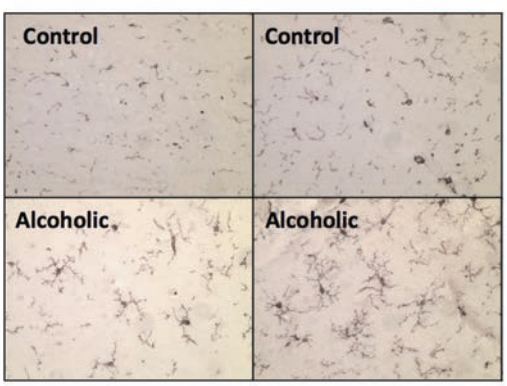

Figure 2 Microglial activation, as indicated by expression of the microglial marker Iba-1, is increased in postmortem alcoholic brain. The photomicrographs depict microglia from postmortem brain samples of alcoholics and control subjects. The number of Iba-1–positive microglia (dark stains) is higher in the alcoholic than in the control samples.

SOURCE: He and Crews 2008.

frontal cortex following a 24-hour withdrawal from chronic ethanol exposure (Pandey et al. 1999, 2001). In addition, neuropeptide Y levels were reduced in the cortex following ethanol treatment, an effect that was accompanied by reduced levels of phosphorylated CREB (Bison and Crews 2003).

The reciprocal relationship between NF-κB and CREB transcription sensitizes neurons to excitotoxicity (Zou and Crews 2006). This reciprocal relationship appears to result from the actions of kinases, such as protein kinase A, which activate CREB transcription but inhibit NF-κB activation. However, the reciprocal relationship also may represent differences between neuronal and glial signaling pathways, because regulation of CREB transcription principally occurs in neurons whereas NF-κB activation of proinflammatory genes primarily occurs in microglia.

In summary, ethanol can directly increase NF-κB–mediated transcription of proinflammatory genes in the brain as well as decrease trophic protective-factor transcription by reducing CREB transcription. Together, these effects decrease the brain's resilience to insults.

Roles of HMGB1 and TLR4

Ethanol induces neuroimmune genes through multiple mechanisms. One mechanism involves alcohol-induced release of HMGB1,[2] which increases NF-κB–mediated transcription of proinflammatory cytokines (Crews et al. 2013; Zou and Crews 2014). Several transmitters and neuroimmune-signaling receptors as well as neuronal excitability increase the release of HMGB1 (Maroso et al. 2010). This protein is a TLR4 agonist that acts through multiple signaling mechanisms in the brain, thereby influencing astrocytes and microglia, as well as neurogenesis, neurite growth, and excitability in adjacent neurons. HMGB1 released by neuronal activity stimulates TLR4 receptors, resulting in IL-1β release and increased phosphorylation of a subunit of the NMDA receptor (i.e., the NR2B subunit), which in turn increases susceptibility to seizures (Maroso et al. 2010; Vezzani et al. 2011). Actively released HMGB1 is acetylated, and ethanol increases HMGB1 acetylation in brain slice cultures. The acetyl-HMGB1 initially is found primarily in the cell's cytosol, likely in vesicles, before its concentration in the surrounding fluid increases progressively, consistent with neuronal release (Zou and Crews 2014). The importance of ethanol-induced release of HMGB1 and resulting TLR4 activation to ethanol-induced neurodegeneration and behavioral pathology was demonstrated in studies using cells and animals that no longer produced TLR4 (i.e., TLR4 knockout cells and mice). The experiments showed that knockout of TLR4 markedly blunted chronic–ethanol-induced neurodegeneration and induction of proinflammatory gene expression (Alfonso-Loeches et al. 2010; Blanco et al. 2005; Fernandez-Lizarbe et al. 2009; Pascual et al. 2011; Valles et al. 2004).

Additional studies found that ethanol treatment induced neuroimmune genes in microglia and astrocyte primary cultures as well as in mice and that this induction was dependent on the expression of TLR4. These receptors are always present on microglia, making microglia a key component of drug-induced neuroimmune activation (Alfonso-Loeches and Guerri 2011; Schwarz and Bilbo 2013). In addition, TLR4 is integral to ethanol-induced dopamine release (Alfonso-Loeches and Guerri 2011), damage to white matter (Alfonso-Loeches et al. 2012), and other pathologies associated with chronic–ethanol-induced changes in the brain (Pascual et al. 2011). In cultured cells, ethanol treatment increases innate immune gene expression in a time-dependent fashion, mimicking responses to LPS or IL-1β administration, although ethanol induces a much smaller response (Crews et al. 2013). In vivo, ethanol induces neuroimmune genes in the brains of wild-type mice, but not TLR4 knockout mice (Alfonso-Loeches et al. 2010). These studies support the hypothesis that TLR4 signaling is critical to many of the effects of alcohol on the brain.

It is not clear why signaling through TLR4 but not via other cytokine receptors seems to contribute significantly to ethanol responses, because all of these receptors generally belong to the same receptor superfamily (i.e., the TLR–IL1-R superfamily) (Wald et al. 2003) and share kinase cascades in monocytes and microglia that all converge upon NF-κB. The findings suggest that the TLR4s on neurons or other brain cells may have some unique properties that differ from NF-κB activation by receptors for TNF-α, IL-1β, and other cytokines (e.g., TNF receptor) which induce NF-κB transcription of proinflammatory cytokines. Further complicating the picture, the TLR4 signaling pathway is not the only one affected by ethanol exposure. Vetreno and colleagues (2013) found that chronic intermittent treatment of adolescent rats also led to persistent increases in the expression of another receptor stimulated by HMGB1, called receptor for advanced glycation end products (RAGE) (see figure 1). Although the mechanisms remain complicated, together these studies suggest that HMGB1–TLR4 and perhaps RAGE signaling (which are found on multiple brain cells types) as well as neuronal–glial neuroimmune signaling and microglial–astrocyte activation all contribute to alcohol-induced brain damage.

Effects of Acute vs. Chronic Ethanol Exposure

Although chronic alcohol treatment increases proinflammatory gene expression in the brain through activation of TLR4, this is confounded by acute alcohol inhibition of TLR4 signaling in monocytes and possibly

[2] HMGB1 also is known as amphoterin (Huttunen and Rauvala 2004).

other cells. Time-dependent acute and chronic opposing effects of ethanol confound many studies (Crews et al. 2006*a*, 2011; Szabo and Mandrekar 2009). Acute ethanol suppresses the innate immune response to LPS, a TLR4 agonist, in both in vivo and in vitro models. For example, LPS-induced TNF-α and IL-1β production is blunted in blood monocytes obtained from healthy human volunteers after acute alcohol exposure (Crews et al. 2006*a*; Szabo et al. 1993, 1995, 2001). In animal models, acute ethanol exposure attenuates the TNF-α, IL-1β, and IL-6 immune responses to LPS (Pruett et al. 2004). Similarly, in in vitro models, addition of ethanol (25 mM) just before LPS blunts induction of TNF-α (Szabo et al. 1993, 1995, 2001). In contrast, chronic in vitro ethanol exposure of astrocytes, microglia, and brain slices induces NF-κB transduction of proinflammatory genes through activation of TLR4 signaling (Crews et al. 2013; Fernandez-Lizarbe et al. 2009; Zou and Crews 2014).

While it is unclear if the presence of acute ethanol exposure antagonizes TLR4 on all cell types, other TLRs are not acutely blocked by ethanol (Crews et al. 2006*a*). Upregulation of TLRs by chronic alcohol treatment can lead to sensitization. In mice, binge treatment with ethanol for 10 days (5 g/kg/day), followed by LPS 24 hours later when alcohol had cleared, resulted in a marked increase in proinflammatory gene induction (Qin and Crews 2012*b*). Ethanol treatment increased the responses to LPS-induced proinflammatory cytokines in liver, blood, and brain. The responses were transient in blood and liver but were long lasting in brain. Similarly, chronic 10-day alcohol treatment sensitized mice to the proinflammatory response to Poly:IC, a compound that activates TLR3 (Qin and Crews 2012*a*). Thus, the effects of ethanol on brain neuroimmune signaling are in part related to increases in TLRs (see figure 1) that increase neuroimmune signaling and cytokines, such as IL-1β, during chronic ethanol treatment, although the presence of alcohol can blunt TLR4 responses during intoxication.

Ethanol Induction of HMGB1–TLR Signaling in the Brain

As mentioned previously, studies investigating the mechanisms of ethanol induction of proinflammatory genes in the brain have shown that chronic ethanol increases expression of TLRs as well as the TLR4 receptor agonist HMGB1. Studies of chronic 10-day ethanol treatment of mice (Crews et al. 2013), chronic in vitro treatment of rat brain-slice cultures (Zou and Crews 2014), and analyses of postmortem human alcoholic brain (Crews et al. 2013) all found increased expression of HMGB1, TLR4, TLR3, and TLR2 (see figure 3).[3] Increases in receptors and agonists are common in innate immune signaling, and these findings suggest that chronic alcohol, through induction of HMGB1 and TLR4 as well as the less well characterized RAGE receptor, may contribute to increases in neuroimmune-gene expression. Brain-slice culture experiments found that ethanol could induce HMGB1 release, which then increased proinflammatory gene expression. This process could be blocked by pharmacological antagonists or knockdown of TLR4 (Crews et al. 2013; Zou and Crews 2014). Studies in adolescent rats (Vetreno and Crews 2012), adolescent mice (Coleman et al. 2014), and adult mice (Qin et al. 2007, 2008, 2013) found long-lasting increases in neuroimmune-gene induction following alcohol treatment.

In humans, levels of HMGB1 and TLR expression in specific brain regions (e.g., the orbitofrontal cortex) have been shown to correlate with lifetime alcohol consumption (Crews et al. 2013) (see figure 4). Alcoholic subjects who vary greatly in the duration and amounts of active drinking bouts exhibited a large variation in lifetime alcohol consumption that correlated with increased HMGB1–TLR expression in the frontal cortex. In contrast, moderate-drinking humans consumed much less alcohol than alcoholics and exhibited much lower HMGB1–TLR expression. This interesting correlation only could occur if ethanol induction of HMGB1–TLR was persistent and cumulative with binge-drinking episodes (see figure 4). Together, these studies suggest that HMGB1–TLR4 signaling is increased by chronic binge drinking, contributing to the persistent and sustained induction of proinflammatory signaling in brain.

[3] Researchers have identified 13 TLRs (i.e., TLR 1-13) in mammals (Medzhitov 2001; Takeda et al. 2003); however, only TLR2, TLR3, and TLR4 have been assessed in alcoholic brain.

Mechanisms of Neurodegeneration Related to Alcohol's Effects on Neuroimmune Signaling in the Brain

Role of NADPH Oxidase and Oxidative Stress

One innate immune gene induced by ethanol and LPS is NADPH oxidase, a multi-subunit enzyme that catalyzes the formation of the reactive oxygen species (ROS), superoxide, and thereby increases oxidative stress. NADPH oxidase first was characterized as a phagocytic oxidase in monocytes, where it was hypothesized to contribute to the oxidation of infectious agents. The superoxide produced by NADPH oxidase can increase NF-κB transcription, thereby creating another amplifying loop of proinflammatory signaling (see figure 1). More recent studies have found that there are multiple genes and forms of NADPH oxidase.

Qin and Crews (2012*b*) discovered that LPS and ethanol can increase expression of NADPH oxidase subunits, particularly the superoxide-forming $gp91^{phox}$ subunit, in the brain and that ethanol treatment of mice increased superoxide formation in

the brain as well as neuronal death. Inhibition of oxidases both reduced superoxide formation and protected against alcohol-induced neuronal death. Other studies in mice demonstrated that LPS treatment induced neuroimmune-gene expression, NADPH-oxidase activity, and oxidative stress that persisted for at least 20 months and led to neurodegeneration (Qin et al. 2013). Prolonged induction of NADPH oxidase and oxidative stress in the brain could contribute to the persistent increase in NF-κB transcription observed after alcohol exposure, because ROS can activate NF-κB. These findings are consistent with the hypothesis that oxidative stress, by inducing innate immune genes, significantly contributes to alcoholic brain damage and alcoholic neurodegeneration.

In addition to enhancing ROS levels, alcohol exposure decreases endogenous antioxidant levels, thereby reducing the body's natural defense against ROS and again increasing oxidative stress (Henderson et al. 1995). Specifically, ethanol decreases the levels of the antioxidant glutathione and the cellular activity of antioxidative enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase. Furthermore, a synthetic superoxide dismutase/catalase mimetic (EUK-134) and a water-soluble analog of vitamin E (Trolox), both of which are well-known antioxidants, protected developing hypothalamic neurons from oxidative stress and cellular apoptosis caused by ethanol-treated microglia medium (Boyadjieva and Sarkar 2013).

Role of Hyperexcitability and Excitotoxicty

Another mechanism contributing to alcoholic neurodegeneration and associated with HMGB1–TLR4 signaling is the excessive stimulation of receptors that results in neuron damage and cell death (i.e., excitotoxicity). Chronic ethanol treatment of neurons leads to increased sensitivity to excitotoxicity (Chandler et al. 1994). This effect primarily involves the neurotransmitter glutamate and its receptors.

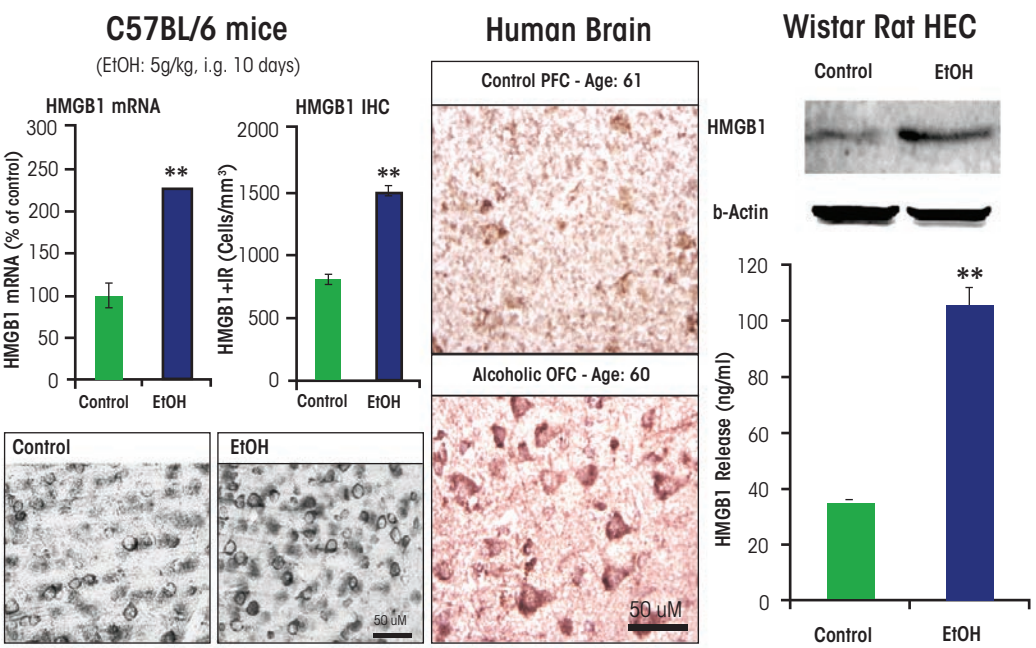

Figure 3 Alcohol increases high-mobility group box 1 (HMGB1) expression in mouse brain, and human brain and induces HMGB1 release from rat brain slices. **(Left)** Chronic ethanol treatment of mice for 10 days increases expression of HMGB1 mRNA and protein. **(Middle)** Postmortem human alcoholic orbitofrontal cortex (OFC) has significantly more HMGB1-immunoreactive cells than seen in age-matched moderately drinking control subjects. **(Right)** Ethanol causes the release of HMGB1 into the media from hippocampal-entorhinal cortex (HEC) slice culture.

NOTE: ** $P < 0.01$, relative to the corresponding control group.
SOURCE: Adapted from Crews et al. 2013.

However, the relationship between ethanol and glutamate receptors is complex. Thus, although ethanol enhances overall glutamate excitotoxicity, in neuronal primary cultures it blocks excitotoxicity associated with a specific type of glutamate receptor (i.e., the NMDA receptor). This is consistent with many studies finding that ethanol inhibits NMDA receptors (Chandler et al. 1998). Yet at the same time, HMGB1–TLR4 signaling (Balosso et al. 2014) and IL-1β receptor signaling (Viviani et al. 2003)—both of which, as described above, are induced by chronic ethanol—increase NMDA receptor-mediated calcium flux, neuronal excitability, and excitotoxicity through activation of kinase signaling cascades, including activation of Src kinase and tyrosine-kinase (see figure 5). Furthermore, Suvarna and colleagues (2005) found that ethanol increases NMDA excitability in the hippocampus through kinase activation that alters receptor trafficking, leading to increased numbers of NMDA receptors containing the NR2B subunit at the synapse.

Another mechanism through which chronic ethanol induces hyperexcitability involves neuroimmune inhibition of glial glutamate transporters (Zou and Crews 2005). Thus, in brain-slice cultures, ethanol potentiates excitotoxicity by causing blockade of the molecules that normally remove glutamate from the synapse into glial cells and may perhaps even induce glutamate release from those cells (Zou and Crews 2006, 2010).

As indicated above, ethanol causes HMGB1 release, creating hyperexcitability that disrupts synaptic plasticity and sensitizes to excitotoxicity. HMGB1 is massively released during brain damage, resulting in persistent neuroimmune-gene induction (Kim et al. 2006). Maroso and colleagues (2010) found that increased HMGB1 release was associated with hippocampal excitability that caused seizures, leading to persistent increases in HMGB1 and excitability. Ethanol has modest cumulative effects with repeated chronic exposure, further exacerbating excitability and excitotoxicity resulting from increased neuroimmune signaling. Thus, the global neurodegeneration associated with alcoholism, with the most severe losses observed in the frontal cortex, is secondary to the persistent and progressive neuroimmune activation.

Neuroimmune-Gene Expression in Postmortem Human Alcoholic Brain

In addition to the HMGB1–TLR4 signaling cascade, multiple other proinflammatory genes are increased and have been detected postmortem in the brains of alcoholics. Initial

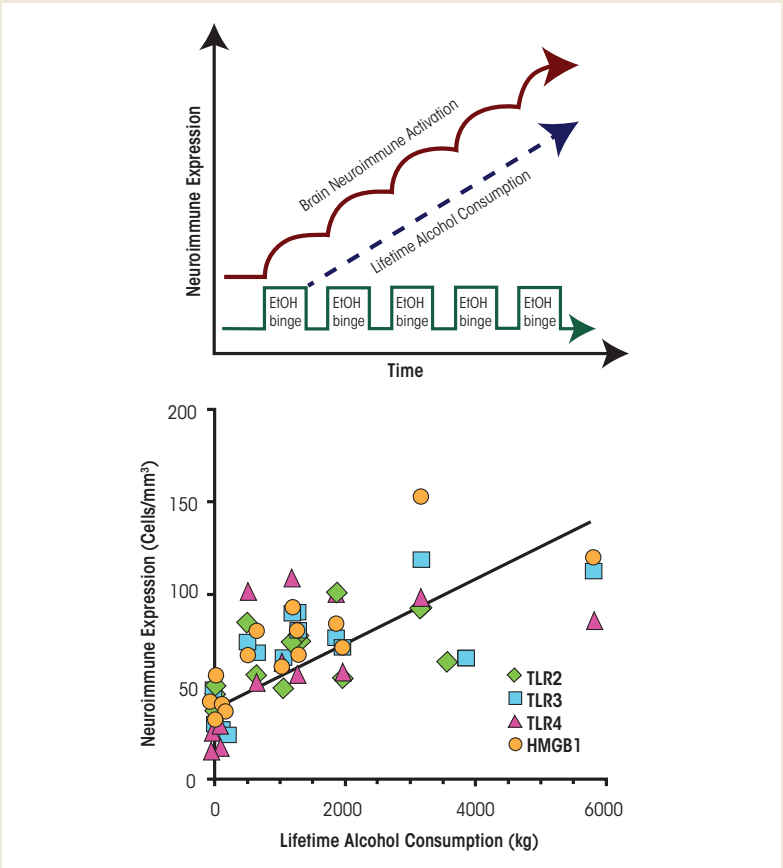

Figure 4 Cycles of chronic alcohol consumption lead to persistently increased neuroimmune-gene expression. **(Top)** Repeated ethanol (EtOH) binges result in increased brain neuroimmune activation (i.e., microglial and astrocytic activation as well as upregulated neuroimmune-gene expression). **(Bottom)** In humans, lifetime alcohol consumption is positively correlated with neuroimmune signal immunoreactivity. Symbols indicate levels of Toll-like receptor (TLR) 2, TLR3, TLR4, and high-mobility group box 1 (HMGB1) in individual moderate drinkers and alcoholics. Results for moderate drinkers are clustered along the Y-axis because of their low lifetime alcohol consumption and similar neuroimmune expression. Alcoholic subjects show a more than 10-fold variation in lifetime alcohol consumption as well as considerable variation in expression of all four neuroimmune genes.

NOTE: Correlations are as follows: TLR2: $r = 0.66$ ($p < 0.01$); TLR3: $r = 0.83$ ($P < 0.001$); TLR4: $r = 0.62$ ($P < 0.01$); HMGB1: $r = 0.83$ ($P < 0.001$).
SOURCE: Crews et al. 2013.

human brain studies focused on microglia and the proinflammatory cytokine monocyte chemotactic protein-1 (MCP-1, also known as CCL2), which among the cytokines tested was induced most robustly by ethanol in brain-slice cultures (Zou and Crews 2012). Additional studies also showed increased levels of MCP-1 protein in the ventral-tegmental area, amygdala, nucleus accumbens, and hippocampus (He and Crews 2008). In addition to MCP-1, expression of the microglial marker Iba-1 also was increased. These studies indicate that neuroimmune-gene expression is increased in the human alcoholic brain.

Subsequent studies focusing on the prefrontal cortex, specifically the orbital frontal cortex (OFC), found increased levels of HMGB1 as well as TLRs (specifically TLR2, TLR3, and TLR4) in postmortem alcoholic brain (Crews et al. 2013). Furthermore, NADPH oxidase was increased in alcoholic OFC, consistent with increased oxidative stress as found in mice. The HMGB1 receptor RAGE also was increased in postmortem human alcoholic brain (Vetreno et al. 2013). Finally, studies detected increased IL-1β inflammasome markers in the hippocampus of postmortem alcoholic brains that could contribute to loss of neurogenesis. These observations indicate that multiple neuroimmune genes are increased in alcoholic brain and likely contribute to neurodegeneration and the neurobiology of alcoholism in humans.

Researchers also investigated the relationship between alcohol drinking and neuroimmune-gene expression in alcoholics and control subjects. Interestingly, two forms of correlations were found linking neuroimmune-gene expression to alcohol consumption and alcoholism. The first correlation involved the age at drinking onset (Vetreno et al. 2013). Adolescent drinking is known to increase risk of developing alcohol dependence, with the risk decreasing with every year of delaying alcohol-use initiation across adolescence (for more information, see the sidebar). Studies found that in the OFC, a negative

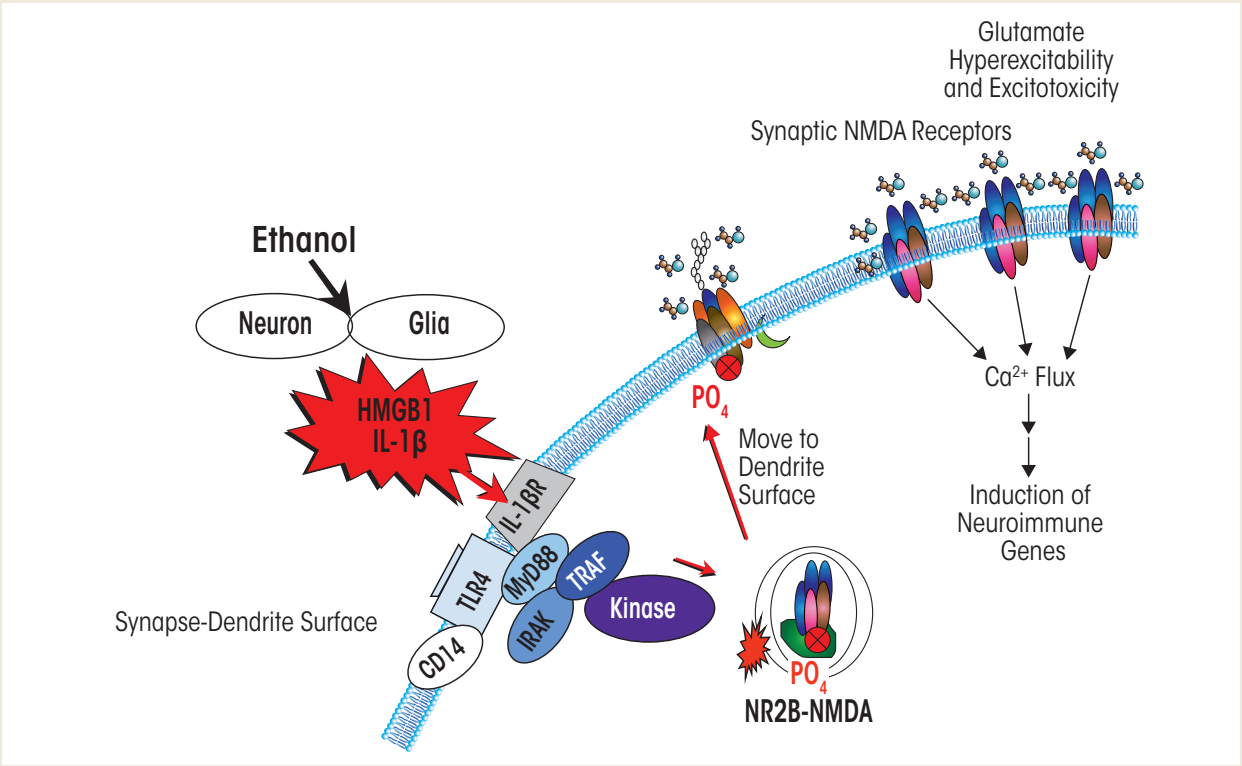

Figure 5 Simplified schematic depicting how neuroimmune signaling leads to neuronal hyperexcitability and the neurobiology of addiction. Alcohol and stress activate neurons and glia in the central nervous system, resulting in the release of various neuroimmune signals (e.g., high-mobility group box 1 [HMGB1] and interleukin-1beta [IL-1β]) that activate neuroimmune receptors (e.g., Toll-like receptors [TLRs]). Neuroimmune receptor stimulation leads to phosphorylation, and thus activation, of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors that are transported to the cell surface (Iori et al. 2013; Maroso et al. 2010). The increased number of NMDA receptors increases Ca^{2+} flux, triggering further induction of neuroimmune genes, and also promotes glutamate hyperexcitability and excitotoxicity.

correlation existed between HMGB1–TLR4 expression and age at drinking onset, with lower HMGB1–TLR4 expression in individuals who initiated alcohol use later. The second correlation involved the amount of alcohol consumed, with total lifetime alcohol consumption positively correlated with OFC expression of HMGB1, TLR4, TLR3, TLR2, and RAGE (Crews et al. 2013). These findings further support the role of neuroimmune signaling in alcoholic brain and alcoholic neurodegeneration.

Role of Microglia in Mediating Alcohol Actions in the Brain

Given their role in facilitating inflammation, it is not surprising that alcohol-activated microglia have been implicated in alcohol-induced inflammatory pathways. In rats, intermittent and chronic alcohol exposure can activate microglia while concomitantly increasing expression of proinflammatory cytokines and neuronal cell death, providing indirect evidence for the role of microglia in alcohol-induced neuroinflammation and neurotoxicity (Alfonso-Loeches and Guerri 2011; Chastain and Sarkar 2014; Zhao et al. 2013). Alcohol can activate microglia directly, via stimulation of TLRs, or indirectly, via neuronal damage and subsequent release of damage-associated molecular patterns that include HMGB1, resulting in the accumulation of microglia in the brain (i.e., reactive microgliosis) (Alfonso-Loeches and Guerri 2011). Microglial TLR4 seems to be necessary in alcohol- induced activation of microglia and subsequent microglial production of inflammatory mediators and apoptosis of neighboring neurons (Fernandez-Lizarbe et al. 2009, 2013). In an in vitro study (Boyadjieva and Sarkar 2010), microglia-conditioned media enhanced ethanol-induced apoptosis of cultured hypothalamic neurons. Interestingly, the neuronal cell death induced by microglia-conditioned media could be abolished if TNF-α was inactivated in the cultured cells, suggesting that microglial TNF-α production plays a key role in ethanol-induced neurotoxicity in developing neurons. The mechanism by which alcohol induces neuronal cell death may involve upregulation of NF-κB expression, which then stimulates release of TNF-α, resulting in neuronal apoptosis (Crews and Nixon 2009; Guadagno et al. 2013). Stimulation of the transcription factor AP-1 and release of IL-1β, IL-6, and transforming growth factor β (TGF-β1) also may contribute to alcohol-induced neuronal apoptosis (Alfonso-Loeches and Guerri 2011; Chen et al. 2006).

In addition to releasing cytokines, stimulated microglia contribute to neurotoxicity by secreting ROS (Takeuchi 2010). ROS, such as superoxide, hydrogen peroxide, and nitric oxide, can break down cell membranes and induce cell death. After alcohol exposure, ROS levels increase both as a natural byproduct of alcohol metabolism and as a result of enhanced cellular respiration, thus creating oxidative stress and leading to neuronal cell death (Guerri et al. 1994; Montoliu et al. 1995). Several studies have implicated microglia in the alcohol-induced production of ROS and resulting neurotoxicity. Qin and Crews (2012*a*) demonstrated that mice exposed to chronic alcohol showed increased levels of NADPH oxidase, superoxide, microglial activation, and cell death in cortical and hippocampal brain regions. Inhibition of NADPH oxidase during alcohol administration decreased superoxide, microglial activation, and cell death, directly linking ROS production to alcohol-induced microglial activation and neurotoxicity. In accord with these in vivo findings, in vitro studies showed that microglia-conditioned media enhanced ethanol-induced ROS production and oxidative stress in cultured hypothalamic neuronal cells and increased apoptotic cell death (Boyadjieva and Sarkar 2013*a*). Through these mechanisms, as well as the ethanol-related decreases in antioxidants discussed earlier, ethanol-activated microglia can induce apoptotic cell death and cell death in cultured fetal hypothalamic neurons from rat, suggesting that microglia may help facilitate ethanol-induced neurotoxicity by ROS.

Another cellular signaling mechanism by which alcohol induces neuronal apoptosis involves increased neuronal release of TGF-β1. Alcohol-induced elevation of TGF-β1 levels in neuronal cells is accompanied by a host of molecular and chemical changes related to cell death, including the following (Chen et al. 2006; Kuhn and Sarkar 2008):

- Increased expression of a protein called E2F1, whose overexpression sensitizes cells to apoptosis;
- Reduced expression of two key regulators of cell-cycle progression (i.e., cyclin D1 and cyclin-dependent kinase-4);
- Elevated levels of mitochondrial proapoptotic proteins bak, bad, and bcl-xs;
- Lowered levels of the antiapoptotic protein bcl-2; and
- Increased production of the apoptotic enzyme caspase 3.

Interestingly, in transformed cells, inhibition of NF-κB or ROS abrogates TGF-β1 stimulation of cell functions (Tobar et al. 2010). Hence, the ROS–NF-κB–TGF-β1 signaling cascade is a possible mechanism by which alcohol induces the apoptotic process in neurons—a process that is modulated by microglia. Another mechanism might relate to the microglial ability to reduce production of BDNF and cyclic adenosine monophosphate (cAMP) in neurons following ethanol activation. Thus, hypothalamic neuronal cell cultures treated with ethanol-activated microglia-conditioned medium showed decreased levels of both of these compounds. Treatment with BDNF or dibutyryl

cAMP decreased the changes in the levels of intracellular free radicals, ROS, nitrite, glutathione, and catalase as well as neuronal apoptotic cell death that otherwise occurred when these cultures were treated with ethanol-activated microglia-conditioned medium. These findings suggest that ethanol increases the production of certain microglia-derived factors, thereby reducing cellular levels of cAMP and BDNF and increasing cellular oxidative stress and apoptosis in neuronal cells (Boyadjieva and Sarkar 2013*b*). However, further studies are needed to fully elucidate the mechanism(s) by which ethanol-activated signaling induces neuronal death.

Microglia also may mediate the effects of alcohol administration on the development of new neurons (i.e., neurogenesis). Alcohol exposure can result in decreased hippocampal neurogenesis, an effect that may underlie alcohol-related neurodegeneration (Crews et al. 2006; Morris et al. 2010). However, alcohol exposure followed by a period of abstinence results in increased hippocampal neurogenesis, which may serve a regenerative purpose. Interestingly, this process is preceded by microglial proliferation, raising the possibility that microglia may facilitate some regenerative mechanisms in recovery from alcohol exposure (McClain et al. 2011; Nixon et al. 2008).

In addition to being implicated in alcohol-induced neurotoxicity, microglia also might contribute to the processes that lead to the development of alcohol use disorder. Recent studies in rodents support a role for microglia in voluntary alcohol drinking and preference. In a quantitative-trait locus analysis of six strains of mice that differ in voluntary alcohol-drinking behavior, alcohol-preferring animals exhibited an increase in the expression of β-2-microglobulin, an NF-κB target gene involved in microglial MHC immune signaling (Mulligan et al. 2006). In addition, knockout of the β-2-microglobulin gene in mice decreased voluntary alcohol consumption and preference (Blednov et al. 2012). Finally, treatment with minocycline, an antibiotic and selective inhibitor of microglia, reduced voluntary alcohol consumption in adult mice (Agrawal et al. 2014). These studies suggest microglia might mediate alcohol preference and might contribute to the development of alcohol use disorder.

Neuroimmune Signaling Integrates CNS Responses to Alcohol and Stress

The Stress Axis and the Peripheral Immune System

Alcohol activation of immune signals and cytokine production in the brain affects not only cellular functions in the brain but also immune-system function in the periphery. The body's main stress response systems—the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS)—are major pathways by which the brain and the immune system communicate. When the HPA axis is activated by a stressful situation, the hypothalamus releases corticotropin-releasing hormone (CRH), which acts on the pituitary to induce the release of adrenocorticotropic hormone. This hormone in turn acts on the adrenal glands to stimulate the release of stress hormones (i.e., glucocorticoids), including cortisol in humans and corticosterone in rodents. These hormones then help coordinate the body's response to the stress. The SNS is part of the autonomic nervous system that regulates the body's unconscious activities to maintain its normal functions. One of the main processes coordinated by the SNS is the fight-or-flight response to stress.

Alcohol has a potent activating effect on the HPA axis as well as on neuroimmune signaling; therefore, these effects may integrate the responses of the central nervous system (CNS) to alcohol and stress (figure 6). For example, TNF-α, IL-1β, and IL-6 act upon the HPA axis and SNS, both directly via local effects and indirectly via the CNS (Besedovsky and del Rey 1996; Pickering et al. 2005; Wilder 1995). Furthermore, CRH has a variety of complex effects on immune cells (Elenkov et al. 1999) and modulates immune/inflammatory responses through receptor-mediated actions of glucocorticoids on anti-inflammatory target immune cells (Tsigos and Chrousos 2002). In contrast, elevated glucocorticoid levels in the prefrontal cortex are proinflammatory, potentiating LPS–TLR4 activation of NF-κB and other proinflammatory signals (Munhoz et al. 2010). The neurotransmitter norepinephrine that is released by SNS activation also disturbs inflammatory cytokine networks and innate immune-cell function. Similarly, the hypothalamic peptide β-endorphin (BEP), whose release is stimulated by CRH during HPA activation, can inhibit stress-hormone production and activate peripheral immune functions (Sarkar and Zhang 2013). All of these findings suggest that the stress–HPA axis, commonly thought to involve anti-inflammatory glucocorticoid actions, also contributes to stress–alcohol responses in the brain that can increase proinflammatory HMGB1–TLR–cytokine signaling.

HPA hormones influence the immune system in multiple ways (figure 6). Glucocorticoids prevent the migration of leukocytes from the circulation into extravascular regions, reduce accumulation of various immune cells (i.e., monocytes and granulocytes), and suppress the production and/or action of many cytokines and inflammatory mediators (Hermann et al. 1995; Sheridan et al. 1998; Zhang et al. 1998). They also inhibit a number of cytokines, including IL-1α, IL-1β, IL-6, IL-12, IFN-γ, TNF-α, granulocyte-macrophage colony-stimulating factor, and chemokine (C-C motif) ligand 5 (RANTES)

Adolescence and Persistent Neuroimmune Expression in the Brain

Adolescence is a developmental stage characterized by increased play behavior, thrill seeking, risk taking, puberty, and transition to independence. During this stage, the brain continues to develop; in particular, the frontal cortex continues to exhibit structural changes that coincide with maturation of adult behaviors and executive functions (Ernst et al. 2009). The developing brain is uniquely sensitive to alcohol, making adolescence a critical period of risk for developing alcohol use disorder (AUD) (Crews et al. 2007). Adolescence also is a period of experimentation, as exemplified by findings that alcohol-use initiation use typically begins during those years. The age of drinking onset is associated with various alcohol-related characteristics, including prevalence of lifetime AUD, as well as violence, fights, and injuries associated with alcohol use (Brown et al. 2008; Dawson et al. 2008; Sher and Gotham 1999). The younger the age of drinking onset, the more likely the person will develop AUD. In addition, binge drinking peaks during late adolescence.

The high prevalence of binge drinking among adolescents increases the importance of understanding how binge drinking might affect the adolescent brain. Studies found that a younger age of drinking onset is associated not only with an increased risk of lifetime AUD but also correlates with a smaller brain size and greater expression of high-mobility group box 1 (HMGB1) and Toll-like receptor 4 (TLR4), as well as other neuroimmune signaling receptors (Vetreno et al. 2013). These associations likely result both from pre-existing conditions that mature into dysfunctional behavior and from alcohol-induced factors than change over the life course and increase dysfunctional behavior, perhaps by altering brain maturation. The contributions of these two factors can only be determined by controlled experiments in which adolescent alcohol exposure is the only variable and genetic and other factors play no role. Such studies cannot be done in humans but are being done in animals (primarily rats) whose genetic background and environment can be controlled. The essential need to understand the neurobiology and impact of adolescent drinking on adulthood resulted in the formation of a consortium called NADIA, funded by the National Institute on Alcohol Abuse and Alcoholism, which addresses the contribution of adolescent alcohol abuse to adult psychopathology.

Adolescents have an immature response to alcohol, characterized by unique factors that differ from the adult response to alcohol. For example, adolescent rats show greater ethanol-induced memory impairment in certain tasks (e.g., the Morris water maze and discrimination tasks) than do adults (Land and Spear 2004; Markwiese et al. 1998). Similarly, humans who initiate alcohol use in their early 20s are more sensitive to the effects of alcohol on multiple memory tasks compared with those who start drinking in their late 20s (Acheson et al. 1998). Also, compared with adults, adolescents exhibit more potent inhibition of NMDA receptor-mediated synaptic activity in the hippocampus (Swartzwelder et al. 1995) as well as greater induction of long-term potentiation (LTP) (Martin et al. 1995). Adolescents, who already exhibit social behaviors, also are uniquely sensitive to the social facilitative effects of ethanol (Varlinskaya and Spear 2002). Consistent with findings in humans, adolescent rats are more sensitive to binge-drinking models of brain damage, particularly in the frontal cortex (Crews et al. 2006). Interestingly, adolescent rats are less sensitive than adults to certain effects of alcohol, such as the sedative (Little et al. 1996; Silveri and Spear 1998), motor impairing (Little et al. 1996, White et al. 2002*a,b*), social inhibitory (Varlinskaya and Spear 2002), and aversive (Anderson et al. 2010) effects. Adolescent rats also show electrophysiological differences from adults in the hippocampus, particularly a reduced sensitivity to γ-aminobutyric acid (GABA) type A (GABAA) receptor-mediated inhibition (Carr et al. 2003; Sullivan et al. 2006; Yan et al. 2009, 2010). The reduced sedative sensitivity to alcohol and increased alcohol-induced cognitive disruption observed in adolescent animals is consistent with findings in humans that adolescents have high rates of binge drinking and are at particularly high risk of alcohol-related traffic crashes. The continuous increase in high binge-drinking levels in human adolescents over the past decade justifies the need to study the long-term consequences of adolescent alcohol abuse in more detail.

Like adult alcohol exposure, adolescent exposure induces neuroimmune genes in the brain; furthermore, in humans, the effect on neuroimmune genes correlates with age of drinking onset. Indeed, Vetreno and Crews (2012) found that intermittent binge-ethanol treatment in adolescent rats increased expression of multiple innate immune genes in the frontal cortex during adulthood. Interestingly, whereas expression of the critical neuroimmune signaling receptor TLR4 decreased during adolescence in controls, expression of this receptor increased and remained elevated into adulthood in adolescents with binge ethanol exposure (Vetreno and Crews 2012). In contrast, the expression of HMGB1 in the frontal cortex

increased during adolescence in control subjects, and this increase was exacerbated by adolescent binge ethanol exposure. Moreover, adolescent alcohol exposure resulted in a persistent increase in adult HMGB1 and TLR4 levels that may represent adolescent-like HMGB1–TLR4 signaling in these adults.

As mentioned in the main article, HMGB1–TLR4 signaling induced by alcohol exposure can enhance sensitivity at the NMDA glutamate receptor, which can counteract ethanol's direct inhibitory effects on this receptor. Accordingly, adults with persistent increases in HMGB1–TLR4 signaling resulting from adolescent alcohol exposure might experience adolescent-like tolerance to alcohol's sedative effects, and perhaps increased adolescent-like cognitive disruption as well. Although adolescent alcohol exposure does not markedly disrupt adult learning tasks, adolescent intermittent binge exposure induces deficits in reversal learning in adult rats (Vetreno and Crews 2012) and mice (Coleman et al. 2011). These studies are consistent with the hypothesis that the adolescent brain is vulnerable to long-lasting changes that persist through maturation into adulthood. Persistent neuroimmune-gene induction likely contributes to continuous slow neurodegeneration as well as to more specific insults on key neurotransmitters that mature during adolescence (Crews et al. 2007; Vetreno and Crews 2012) and may also be related to a persistent loss of behavioral flexibility. Together, the persistent loss of ability to adapt to changes, low sedative response to alcohol, and increased sensitivity to cognitive disruption associated with adolescent alcohol exposure all are likely to promote and sustain high alcohol-drinking levels. These in turn will promote more alcohol consumption and the chances that AUD will develop in addition to alcoholic neurodegeneration.

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(Sapolsky et al. 2000; Wiegers et al. 2005). At the same time, glucocorticoids increase the activity of TGF-β by activating a latent form of the cytokine (Oursler et al. 1993), which may indirectly affect the immune response, because TGF-β inhibits activation of T cells and macrophages. Glucocorticoids also increase production of IL-10, an anti-inflammatory cytokine that blocks NF-κB transcription and inhibits antigen presentation and T-cell activation (de Waal Malefyt et al. 1991). Finally, glucocorticoids suppress maturation, differentiation, and proliferation of immune cells, including innate immune cells, T cells, and B cells.

Norepinephrine released after SNS activation also disturbs inflammatory cytokine networks by inhibiting production of immune-enhancing cytokines, such as IL-12 and TNF-α, and by upregulating production of inhibitory cytokines, such as IL-10 and TGF-β (Webster et al. 2002). Additionally, norepinephrine affects peripheral natural killer (NK) cells, a subset of lymphocytes that are a first-line defense against viral infections, tumor growth, and metastasis via their unique cytolytic action (Herberman and Ortaldo 1981). These cells carry receptors for norepinephrine (i.e., β-adrenergic receptors) on their surfaces (Madden et al. 1995). The cytolytic activity of NK cells involves the synergistic actions of the pore-forming protein perforin and the serine protease granzyme B to cause apoptosis of target cells (Graubert et al. 1996). Among the HPA hormones, glucocorticoids and CRH both are potent inhibitors of NK-cell activity in vitro and in vivo. Hypothalamic CRH inhibits NK activity and IFN-γ production through actiavation of the SNS, which causes release of catecholamines (e.g., norepinephrine) from the spleen and activation of β-adrenergic receptors on NK cells (Irwin et al. 1990). Thus, it appears that the hormones secreted during stress by the HPA axis and SNS have inhibitory effects on peripheral immune functions that contrast with their actions in the CNS.

During activation of the HPA axis, secretion of CRH and catecholamines also increases the secretion of BEP in the hypothalamus. In a feedback mechanism, BEP regulates the secretion of CRH in a hypothalamic region called the paraventricular nucleus (PVN) (Plotsky 1986). In the PVN, the BEP-releasing neurons act on CRH-releasing neurons and inhibit CRH release, thus regulating the activity of the stress system (Plotsky et al. 1993). BEP acts by binding to δ- and μ-opioid receptors; accordingly, treatment with a μ-opioid receptor antagonist results in increased CRH release (Boyadjieva et al. 2006). BEP affects immune-system function through a variety of mechanisms. By binding to δ- and μ-opioid receptors BEP modulates neurotransmission in sympathetic and parasympathetic neurons via neuronal circuitry within the PVN, ultimately resulting in activation of NK-cell cytolytic functions in the spleen (Boyadjieva et al. 2006, 2009; Sarkar et al. 2011). If incubated with human bone marrow mononuclear cells or NK-enriched cell populations, BEP enhances NK activity (Mathews et al. 1983). In animal models, chronic BEP infusion into the blood vessels in the brain enhances NK-cell activity in vivo, and this effect is eliminated by the opioid antagonist naloxone (Jonsdottir et al. 1996). BEP also can inhibit T-cell proliferation (van den Bergh et al. 1993) as well as antibody production (Morgan et al. 1990).

Abnormalities in BEP neuronal function are correlated with a higher incidence of cancers and infections in patients with schizophrenia, depression, and fetal alcohol syndrome and in obese patients (Bernstein et al. 2002; Lissoni et al. 1987; Polanco et al. 2010; Zangen et al. 2002). Interestingly, BEP-cell transplantation in the hypothalamus suppresses various cancers in rat models by activating innate immune-cell functions and altering inflammatory and anti-inflammatory cytokine milieus (Sarkar et al. 2008). In this setting, chronic alcohol use suppresses BEP neuronal activity and is connected with increased infection rates and higher incidence of cancer. Thus, alcohol and stress seem to paralyze adaptive innate immune functions by inducing complex changes in NK cells and other adaptive immune signaling that in the brain primarily involves microglial–astrocyte–neuronal HMGB1–TLR signaling.

Effects of Immune System Activation on Brain Function

The interaction between the brain and the immune system is not unidirectional—that is, immune-system responses also may influence responses in the brain. Recent studies indicate that ethanol causes HMGB1 release in the gut, which activates TLR4. As a result, the gut leaks LPS-like bacterial products, thereby stimulating proinflammatory cytokine induction in the liver, which in turn leads to increased levels of TNF-α and other cytokines in the blood. Qin and Crews (2007, 2012*b*) discovered that LPS-induced increases in serum TNF-α as well as proinflammatory cytokines led to gene induction in the brain. The proinflammatory cytokines in the blood can be transported across the blood–brain barrier (BBB) by their receptors (e.g., TNFR) (Banks and Erickson 2010; Qin et al. 2007). Using intraperitoneal injections of LPS to stimulate proinflammatory responses in the liver and other tissues and induce proinflammatory cytokines, researchers discovered parallel increases in TNF-α in the blood and brain (Qin et al. 2007). In transgenic mice lacking TNF receptors, however, LPS increased TNF-α only in the blood but not in the brain, suggesting that LPS–TLR4 induction of TNF-α in the blood leads to TNF transport by its receptors across the BBB and activation of proinflammatory responses in the brain. Transgenic

mice without the TNF-α receptor cannot transport the cytokine to the brain; consequently, the LPS–TLR4 proinflammatory response is amplified across peripheral tissues but does not spread to the brain.

Ethanol can increase proinflammatory cytokine levels in the blood by activating proinflammatory responses in the liver and other tissues. One mechanism seems to involve the ethanol-induced increase in gut permeability (or "leakiness") mentioned above (Ferrier et al. 2006). At high doses (at least 2 to 3 g/kg ethanol administered into the stomach), ethanol potentiates innate immune signaling in the gut (Ferrier et al. 2006). This disrupts the

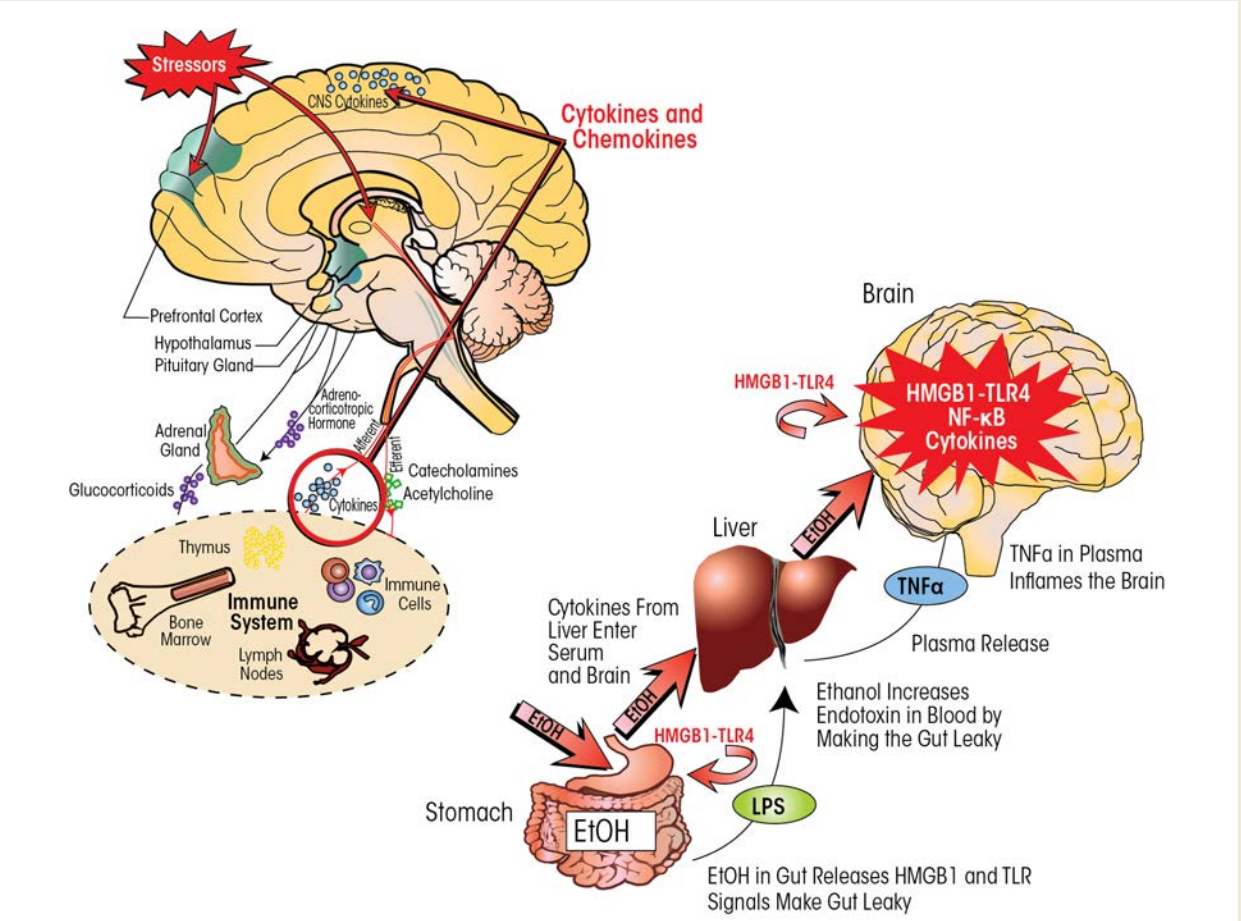

Figure 6 Neuroimmune signaling integrates central nervous system (CNS) responses to alcohol and stress. **(Left)** Stressors activate the body's stress response system, which is comprised of the hypothalamus, pituitary gland, and adrenal glands (i.e., HPA axis) as well as the stress hormones they produce (e.g., adrenocorticotropic hormone and glucocorticoids). Stress also activates the sympathetic nervous system, which secretes catecholamines. These hormones act on various organs and tissues that are part of the immune system. In response, immune cells secrete cytokines that via the blood are transported to the brain. There, these cytokines lead to brain neuroimmune-gene induction that sensitizes stress-response pathways. At the same time, the immune system communicates with the CNS through sensory (afferent) nerves that activate the brain in response to stressful stimuli. This communication pathway involves particularly the vagus nerve and the nucleus tractus solitarius in the brain stem. **(Right)** Alcohol influences neuroimmune signaling via its effects on the gastrointestinal tract. Consumed ethanol enters the stomach and gut and makes them "leaky" by inducing the release of high-mobility group box 1 (HMGB1), which in turn activates Toll-like receptor 4 (TLR4) in the gut. As a result, bacterial products such as lipopolysaccharide (LPS) can enter the blood and reach the liver. Both LPS and ethanol (which also reaches the liver via the circulation) contribute to inflammatory reactions in the liver, which lead to release of tumor necrosis factor-alpha (TNF-α) and other proinflammatory cytokines from the liver. These proinflammatory cytokines in the blood enter the brain and increase neuroimmune-gene expression. Chronic ethanol also increases expression of HMGB1–TLR4 signaling in the brain, leading to persistent and progressive increases in neuroimmune-gene expression in the brain.

connections between the cells lining the gut (i.e., gut tight junctions) and opens sites that allow gut bacteria and their endotoxins to enter the blood vessels leading to the liver, where they can initiate a proinflammatory response (Sims et al. 2010). Thus, high doses of ethanol increase systemic proinflammatory responses, which can then spread to the brain through TNF-α and likely other cytokines (see figure 6).

Although some in vitro studies have suggested that ethanol can interfere with the BBB, most in vivo studies do not show BBB damage following chronic ethanol treatment. Marshall and colleagues (2013) assessed BBB integrity by tracking a protein (i.e., albumin) that cannot cross an intact BBB in rats that were administered large amounts of alcohol for 4 days (a regimen that can induce alcoholic brain damage). The analyses found no evidence of albumin in the brain, indicating that the BBB had remained intact following the ethanol treatment. Using the same model, Crews and colleagues (2006) found that inhibition of NF-κB protected against the brain damage and inhibition of neurogenesis normally induced by this regimen. These findings are consistent with the assumption that proinflammatory responses in the brain mediate brain damage without causing BBB damage. Instead, the brain damage may be induced through direct activation of proinflammatory responses in the brain and/or systemic proinflammatory signals that are transported across the BBB and contribute to brain proinflammatory responses.

Although the levels of proinflammatory gene expression in the blood and brain parallel each other at early time points after initiation of an immune response, the brain's response to LPS is much smaller than that found in the liver and blood during the first few hours. Surprisingly, the blood and liver responses to LPS return to baseline over about 8 to 12 hours, whereas the increase in proinflammatory gene

Glossary

Antibody: Immune molecule (protein) produced by B cells that recognizes foreign molecules that have entered the body (i.e., antigens), binds to these molecules, and marks them for destruction by the body's immune system.

Astrocytes: Characteristic star-shaped non-neuronal cells in the brain and spinal cord that support the endothelial cells that form the blood–brain barrier and provide nutrients to the nervous tissue.

Cytokine: Any of a group of molecules, produced primarily by immune cells, that regulate cellular interactions and other functions; many cytokines play important roles in initiating and regulating inflammatory reactions.

Endotoxin: A highly toxic chemical component of the cell walls of certain bacteria that occur normally in the intestine. Endotoxin can be released into the bloodstream when bacteria die or there is an increase in gut permeability.

Excitotoxicity: Pathological process by which nerve cells are damaged or killed after being excessively stimulated by excitatory neurotransmitters (e.g., glutamate).

Kinase: An enzyme that transfers phosphate groups from one molecule (the donor) to a specific target molecule (the substrate).

Long-term potentiation (LTP): Process by which an episode of strong receptor activation at a synapse leads to a subsequent long-lasting strengthening of the signal transmission across that synapse (e.g., by inducing the accumulation of more receptor molecules at that synapse).

Macrophages: A type of immune cell that ingests foreign particles and microorganisms in a process called phagocytosis and which synthesizes *cytokines* and other molecules involved in inflammatory reactions.

Major histocompatibility complex (MHC): A highly diverse set of glycoproteins in the cell membranes of almost all cells that help to present foreign molecules (i.e., antigens) to other immune cells (i.e., T cells) to activate these cells and induce an immune response.

Microglia: Type of non-neuronal cell in the central nervous system (CNS) that acts as the first and main form of active immune defense in the CNS.

Monocytes: A type of white blood cell involved in the innate immune response; upon activation (e.g., in response to an infection) they move to the site of the infection, enter the tissues, and differentiate into *macrophages*, which then can engulf and destroy the pathogen.

Sepsis: The presence of pathogenic organisms or their toxic products in the blood or tissues.

Toll-like receptors (TLRs): A class of proteins that play a key role in innate immunity. They are located on *macrophages* as well as other brain cells (i.e., *astrocytes* and neurons) and are activated in response to various pathogens; this activation triggers additional innate immune responses and, eventually, adaptive immune responses.

expression in the brain persists for months. This leads to degeneration of dopamine neurons in the substantia nigra, a region in the midbrain involved in reward and addiction (Qin et al. 2007). Similarly, liver and blood responses to binge alcohol exposure appear to be small and transient, although they have not been extensively investigated. In contrast, brain expression of the proinflammatory cytokine MCP-1 persists for at least 1 week (Qin et al. 2008).

Exposure of C57BL/6 mice to 10 daily doses of ethanol followed by LPS results in increased LPS induction of proinflammatory cytokines in the liver, blood, and brain compared with control animals treated only with LPS (Qin et al. 2008). However, this ethanol-induced sensitization to the LPS response resulted in sustained increases in multiple proinflammatory cytokines, including TNF-α, IL-1β, and MCP-1 only in the brain, but not in the liver. The mechanism underlying the sustained brain response and transient liver response is not clear. The investigators noted that the anti-inflammatory cytokine IL-10, which inhibits NF-κB, was increased in the liver 1 week after alcohol treatment, but decreased in the brain (Qin et al. 2008). This suggests that anti-inflammatory mechanisms may contribute to the loss of the liver response. Further analyses found that mice pretreated with ethanol are sensitized not only to the TLR4 receptor agonist LPS but also to the TLR3 agonist Poly:IC (Qin and Crews 2012*a*). Similar to LPS, Poly:IC induces proinflammatory genes in the brain at 24 hours after 10 days of daily alcohol administration (5 g/kg/day). These findings suggest that chronic ethanol sensitizes proinflammatory TLR responses that are easily observed after the clearance of alcohol.

Taken together, the observations indicate that chronic ethanol sensitizes both systemic and brain responses to neuroimmune-gene activation through induction of HMGB1 and TLR proteins. Ethanol-induced leaky gut occurs after high binge-drinking doses, with gut ethanol exposure often being equivalent to the beverage content (i.e., 80 proof is 40 percent ethanol). As a result, bacterial products enter the circulation to the liver and activate liver monocytes (i.e., Kupffer cells), which then produce cytokines, including TNF-α. The TNF-α can be transported to the brain, activating brain neuroimmune signaling that persists for long periods (Qin et al. 2007). Thus, at least two mechanisms of ethanol activation of neuroimmune signaling exist—a direct activation within the brain and the spread of a systemic innate immune activation to the brain (figure 6).

Summary

Binge drinking stimulates neuroimmune-gene induction, which increases neurodegeneration through increased oxidative stress, particularly NADPH oxidase-induced oxidative stress. In addition, HMGB1–TLR4 and NF-κB signaling are increased, leading to enhanced expression of NF-κB target genes and, ultimately, to persistent and sensitized neuroimmune responses to ethanol and other agents that release HMGB1 or directly stimulate TLR receptors and/or NMDA receptors. Persistent neuroimmune-gene induction alters stress-coping mechanisms and the sympathetic nervous system, resulting in the HPA-mediated enhancement of peripheral cytokines, which further exacerbates the neuroimmune response. In addition to neuroimmune signaling, glutamate excitotoxicity also is linked to alcoholic neurodegeneration.

It has been proposed that, instead of simply being a side effect of excessive alcohol consumption, neuronal damage associated with drinking actually may underlie some of the mechanisms of developing alcohol use disorder (Crews and Boettiger 2009). The development of dependence is thought to result at least in part from a lack of inhibition of the subcortical mesolimbic reward system by the frontal cortex (Koob and Le Moal 1997). Alcohol-induced cell death in regions such as the prefrontal cortex may lead to lack of inhibition in subcortical reward areas such as the striatum, which in turn may reduce behavioral inhibition and increase motivation to drink. Repeated stimulation of the innate immune system during chronic or heavy alcohol consumption may facilitate this process, leading to decreased inhibition of the mesolimbic reward system and thus increased drinking (Crews et al. 2011). These processes may be particularly relevant in adolescence, when persistent and long-lasting increases in brain neuroimmune-gene expression and neurodegeneration may be associated with the development of alcohol use disorder.

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The authors declare that they have no competing financial interests.

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Alcohol's Burden on Immunity Following Burn, Hemorrhagic Shock, or Traumatic Brain Injury

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Alcohol consumption contributes to increased incidence and severity of traumatic injury. Compared with patients who do not consume alcohol, alcohol-consuming patients have higher rates of long-term morbidity and mortality during recovery from injury. This can be attributed in part to an impaired immune response in individuals who consume alcohol. Acute and chronic alcohol use can affect both the innate and adaptive immune defense responses within multiple organ systems; the combination of alcohol use and injury results in increased susceptibility to bacterial and viral pathogens. This review examines the major deleterious effects of alcohol on immunity following tissue damage or traumatic injury, with a focus on alcohol's influence on the ability of the immune and major organ systems to fight disease and to repair damaged tissues following injury.

Key words: Alcohol consumption; alcohol use, abuse, and dependence; chronic alcohol use; acute alcohol use; injury; traumatic injury; morbidity; mortality; immune response; impaired immune response; bacterial pathogens; viral pathogens; tissue; organs; disease

The incidence of traumatic injury in alcohol-intoxicated individuals continues to escalate. According to the Centers for Disease Control and Prevention (2012*a*), more than 38 million American alcohol users consume 5 or more drinks on the same occasion (i.e., binge drink) and do so about 4 times per month. This behavior is highly conducive to unintentional or accidental traumatic injury, which according to the National Vital Statistics Reports is the leading cause of years of potential life lost (YPLL) before age 45. Unintentional injury causes more YPLL than that attributed to cancer, intentional injuries, heart disease, and HIV individually (Centers for Disease Control and Prevention 2009). Data from the National Center for Injury Prevention and Control, as well as data derived from prospective and retrospective studies, show that up to 40 percent of victims of traumatic injury have positive blood alcohol concentrations (BAC), with 35 percent presenting with blood alcohol levels above the legal limit of intoxication (Beech and Mercadel 1998).

The severity of trauma, reduced blood flow and oxygen delivery (i.e., hemorrhagic shock, referred to as shock in this article), and tissue injury is greater in intoxicated victims than in sober victims, resulting in higher mortality rates in the alcohol-consuming patient population (Pories et al. 1992). Although immediate mortality from traumatic injury has improved significantly as a result of aggressive resuscitation, long-term morbidity and mortality continue to be unacceptably high during the recovery period. The prevalence of morbidity and mortality is particularly attributable to the altered immune response among impaired patients to subsequent challenges, such as surgery or infection, leading to multiple organ failure (Roumen et al. 1993; Sauaia et al. 1994). Acute alcohol intoxication complicates the initial management of trauma victims and is associated with greater incidences of pneumonia and respiratory distress, requiring ventilator assistance during hospitalization (Gurney et al. 1992; Jurkovich et al. 1992). In addition, major complications including tracheobronchitis, pneumonia, pancreatitis, and sepsis are significantly

increased in patients with high levels of carbohydrate-deficient transferrin (CDT), a marker for alcoholism (Spies et al. 1998). European studies show that, compared with nonalcoholics, alcoholics more frequently develop major complications and require a significantly prolonged stay in the intensive care unit (ICU) following trauma (Spies et al. 1996*a*).

Excessive acute and chronic alcohol consumption has significant effects at multiple cellular levels, affecting both innate and adaptive immune mechanisms (Molina et al. 2010). Both chronic and acute patterns of alcohol abuse lead to impaired immune responses, resulting in increased susceptibility to infectious diseases caused by bacterial and viral pathogens (Brown et al. 2006). Clinical and preclinical studies show that the combined effects of alcohol and injury result in greater immune disruption than either insult alone (Messingham et al. 2002). This article reviews the current understanding of the burden of alcohol on the immune response to three specific traumatic events: burn, shock, and traumatic brain injury (TBI). The major pathophysiological consequences of these injuries on other major organ systems—including the cardiovascular system, pulmonary system, and gastrointestinal tract—are highlighted with emphasis on the contribution of alcohol-induced immunomodulation to postinjury morbidity.

Reestablishment of homeostasis after a traumatic insult involves activation of host defense mechanisms for self-protection against toxic inflammatory processes and tissue repair. Trauma victims frequently are subjected to necessary invasive procedures, such as surgery and anesthesia. In addition, trauma victims frequently are exposed to subsequent challenges, particularly infection. These additional stresses to an already compromised inflammatory and neuroendocrine milieu further contribute to morbidity and mortality in this patient population. Traumatic injury and hemorrhagic shock produce a temporal pattern with early upregulation of pro-inflammatory cytokine[1] gene product expression and with later suppression of stimulated pro-inflammatory cytokine release (Hierholzer et al. 1998; Molina et al. 2001). Together, these alterations lead to generalized immunosuppression, ultimately resulting in an increased susceptibility to infection (Abraham 1993; Ertel et al. 1993).

Alcohol has been shown to affect multiple aspects of the host immune response, contributing to pathological processes (Szabo 1998). For example, alcohol alters the expression and processing of cytokines and a type of cytokine known as chemokines (D'Souza et al. 1989; Standiford and Danforth 1997), the expression of adhesion molecules (Zhang et al. 1999), inflammatory cell recruitment (Patel et al. 1996; Shellito and Olariu 1998) and accumulation, and oxidative capacity of macrophages (Nilsson and Palmblad 1988). The monocyte/macrophage production of cytokines and chemokines, in particular interleukin (IL)-8 and tumor necrosis factor-α (TNF-α), is critical in the regulation of the acute inflammatory host response to infectious challenge. The combined inhibition of pro-inflammatory cytokine production and neutrophil activation and migration to a site of infection has been suggested to contribute to the enhanced susceptibility to infection in alcoholic individuals (Nelson et al. 1991) and to the increased risk of trauma- and burn-related infections associated with alcohol intoxication (Arbabi et al. 1999). Several lines of evidence show that these alcohol-mediated alterations in host defense following injury lead to increased morbidity and mortality from infections during the recovery period (Faunce et al. 2003; Messingham et al. 2002; Zambell et al. 2004). In addition, considerable evidence suggests that the severity of disease processes is greater in intoxicated trauma victims than in nonintoxicated counterparts (Spies et al. 1996*a,b,* 1998). In particular, immunoparalysis characterized by inhibition of stimulated pro-inflammatory cytokine release (Angele et al. 1999) and alterations of both cellular and humoral immunity (Napolitano et al. 1995; Wichmann et al. 1998) have been identified as risk factors for infection and progression to organ injury during the post-traumatic injury period (Abraham 1993; Ertel et al. 1993).

The systemic response to injury is associated with marked activation of neuroendocrine pathways that contribute to cardiovascular adaptation to blood loss, injury, and pain but also exert immunomodulatory effects (Molina 2005). Catecholamines (e.g., dopamine, norepinephrine, and epinephrine), and drugs that mimic their effects (i.e., adrenergic agonists), are especially known to exert important regulatory functions on macrophages as well as on B- and T-lymphocyte cytokine production, proliferation, and antibody secretion; dendritic cell function; cytokine and chemokine release; and nitric oxide (NO) production (Madden et al. 1995). The relevance of these control mechanisms and the implications of their dysregulation have been demonstrated by the high incidence of infection in patients who experience elevated temperature, increased heart rate, and perspiration (i.e., "sympathetic storm") following acute brain trauma and myocardial infarction (Woiciechowsky et al. 1998). Alcohol intoxication produces marked disruption of several neuroendocrine pathways. Disruption of the homeostatic neuroendocrine counterregulatory response to shock impairs hemodynamic stability and recovery, contributing to compromised blood flow and increased end-organ injury (Molina et al. 2013). Specifically, binge alcohol use blunts central neuroendocrine and autonomic activation, and this seems to result from alcohol-accentuated NO production in the periventricular nucleus (PVN) of the hypothalamus (Whitaker et al. 2010). Alcohol-mediated impairment of neuroendocrine counterregulatory responses to traumatic injury not only

[1] Cytokines are proteins involved in cell signaling. They are produced by a variety of cells including immune cells and regulate the immune response.

exacerbates low blood pressure (i.e., hypotension) during hemorrhage but also attenuates blood pressure recovery during fluid resuscitation, leading to significant alterations in blood flow redistribution and notably affecting circulation in the gastrointestinal tract (Wang et al. 1993). Studies have shown that alcohol-intoxicated animals have greater reduction of blood flow to the liver, kidney, and small and large intestines than nonintoxicated animals, following shock and fluid resuscitation (Sulzer et al. 2013). These macro- and microcirculatory changes during trauma and hemorrhage have been implicated in the subsequent development of sepsis and multiple organ failure (Peitzman et al. 1995) and contribute to an increased host susceptibility to infection and tissue injury during recovery (Mathis et al. 2006; Xu et al. 2002). People who abuse alcohol, including both binge and chronic drinkers, have a higher incidence of traumatic injury such as burn, shock, and TBI. The host response to these diverse insults is markedly affected by both patterns of alcohol abuse and some systems—including gastrointestinal, cardiovascular, and pulmonary—are more affected than others according to the specific injury.

Alcohol and Burn Injury

Burn injury is a common type of traumatic injury that affects thousands of people in the United States every year (Bessey et al. 2014). Approximately 50 percent of burn-injured patients have detectable blood alcohol levels at the time of hospital admission (Haum et al. 1995; McGwin et al. 2000), and these patients have more complications, require longer hospital stays, and have greater mortality rates than those with a similar degree of injury who are not intoxicated at the time of injury (McGill et al. 1995). Most morbidity and mortality among patients who survive initial injury is attributed to complications stemming from infection (Baker et al. 1980). Therefore, the pre-burn immunological condition of injured patients affects susceptibility to infection and survival. Several mechanisms contribute to infection in burn patients, including loss of barrier function, changes in normal flora, wound ischemia, and cellular immunosuppression resulting from pro-inflammatory processes. Neutrophil, helper T-cell, and macrophage dysfunction; increased pro-inflammatory cytokine production; and enhanced production of immunosuppressive factors have all been shown to contribute to the pathophysiological response to burn injury (Faunce et al. 1998; Messingham et al. 2000). The mechanisms that contribute to infection in burn patients are influenced by acute and chronic alcohol intoxication and will be discussed below (see figure 1).

Research by Kovacs and colleagues (2008) has offered insight into the combined effects of burn injury and alcohol intoxication on immunity (Bird and Kovacs 2008). Chronic alcohol abuse alone increases the risk for lung infection (Baker and Jerrells 1993), impairs the phagocytic activity of alveolar macrophages and clearance of infectious particles from the airways, and impairs oxidant radicals, chemokine, and cytokine release that are required for microbial killing (Brown et al. 2007; Mehta and Guidot 2012; Molina et al. 2010). Acute alcohol intoxication prior to burn injury significantly suppresses the immune response relative to the insult alone (Faunce et al. 1997) and causes greater suppression of T-cell proliferation and response, reduced IL-2 production, and increased IL-6 production and circulating levels (Choudhry et al. 2000; Faunce et al. 1998). The T-cell and cytokine impairment caused by the combined effect of alcohol and burn injury may further suppress cell-mediated immunity, resulting in even greater susceptibility to infection than burn alone. Alcohol-mediated immunomodulation contributes to tissue injury in target organs as described below.

Gastrointestinal Tract

A multitude of studies have demonstrated that the gut is a reservoir for pathogenic bacteria, which may contribute to increased susceptibility to infections following traumatic injury (Deitch 1990). The intestinal mucosal barrier serves a major role in the local defense against bacterial entry and the translocation of endotoxin to the systemic circulation (Xu et al. 1997). Increased permeability and immune dysfunction indicate the compromised state of the intestinal mucosal barrier to bacterial translocation following trauma (Deitch et al. 1990; Willoughby et al. 1996). Increased intestinal permeability enhances bacterial and endotoxin translocation from the intestinal tract to the systemic circulation, triggering a systemic inflammatory response (Xu et al. 1997). Activated macrophages and lymphocytes release pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, thereby contributing to tissue injury (Fink 1991). Studies have determined that chronic alcohol consumption disrupts intestinal barrier function and induces gut leak (Li et al. 2008; Tang et al. 2009). In addition, reports have shown a loss of intestinal barrier function followed by an increase in endotoxin and bacterial translocation to the systemic circulation following burn injury alone (Carter et al. 1990; Deitch and Berg 1987; Horton 1994), alcohol intoxication alone (Keshavarzian et al. 1994; Tabata et al. 2002), and burn injury with alcohol intoxication (Choudhry et al. 2002; Kavanaugh et al. 2005; Napolitano et al. 1995). Acute alcohol intoxication at the time of burn injury enhances bacterial growth in the intestine and is reflected in a proportional increase in mesenteric lymph node bacterial count (Kavanaugh et al. 2005). Acute alcohol intoxication also modulates intestinal immune defense by suppressing T-cell proliferation and increasing bacterial accumulation in mesenteric lymph nodes, spleen, and blood, which suggests that T-cell suppression may play a role

in bacterial translocation from the lumen of the gut (Choudhry et al. 2002). Moreover, studies have shown that following shock, trauma, or burn injury, the gut leaks bacteria and pro-inflammatory factors that are carried by the mesenteric lymphatic system, which contributes to acute lung injury (ALI) (Magnotti et al. 1999). The possibility that alcohol exacerbates toxin delivery to the systemic circulation through the lymphatics is supported by studies demonstrating that alcohol regulates the contractile cycle of mesenteric lymphatic vessels modulating the driving force of lymph flow (Keshavarzian et al. 1994; Souza-Smith et al. 2010). Thus, the contribution of gut–lymph to end-organ damage following burn injury and alcohol intoxication may be significant.

Collectively, studies indicate that alcohol consumption preceding burn injury (1) increases gut permeability; (2) enhances intestinal bacterial growth, translocation, and systemic accumulation; and (3) suppresses T-cell proliferation. Further, research supports the concept that the intestine is not only a source of infection but also the site of the initial immune perturbation leading to the development of multiple organ dysfunction or organ failure.

Cardiovascular System

Immediately following a burn injury, the cardiovascular system responds with a decrease in cardiac output (Cuthbertson et al. 2001) as a result of low blood volume and reduced venous return (Kramer et al. 2007). This phase is associated with decreased cardiac contractility, mediated by the release of vasoactive and pro-inflammatory mediators (Williams et al. 2011). Subsequently, there is a surge in counterregulatory neuroendocrine mediators (catecholamines, glucagon, and cortisol) that contribute to the development of a hyperdynamic cardiovascular state—characterized by increased heart rate and cardiac output—and is associated with increased myocardial oxygen consumption and myocardial hypoxia (Williams et al. 2011). These pathophysiological processes enhance oxidative metabolism and increase the risk for free-radical generation, further

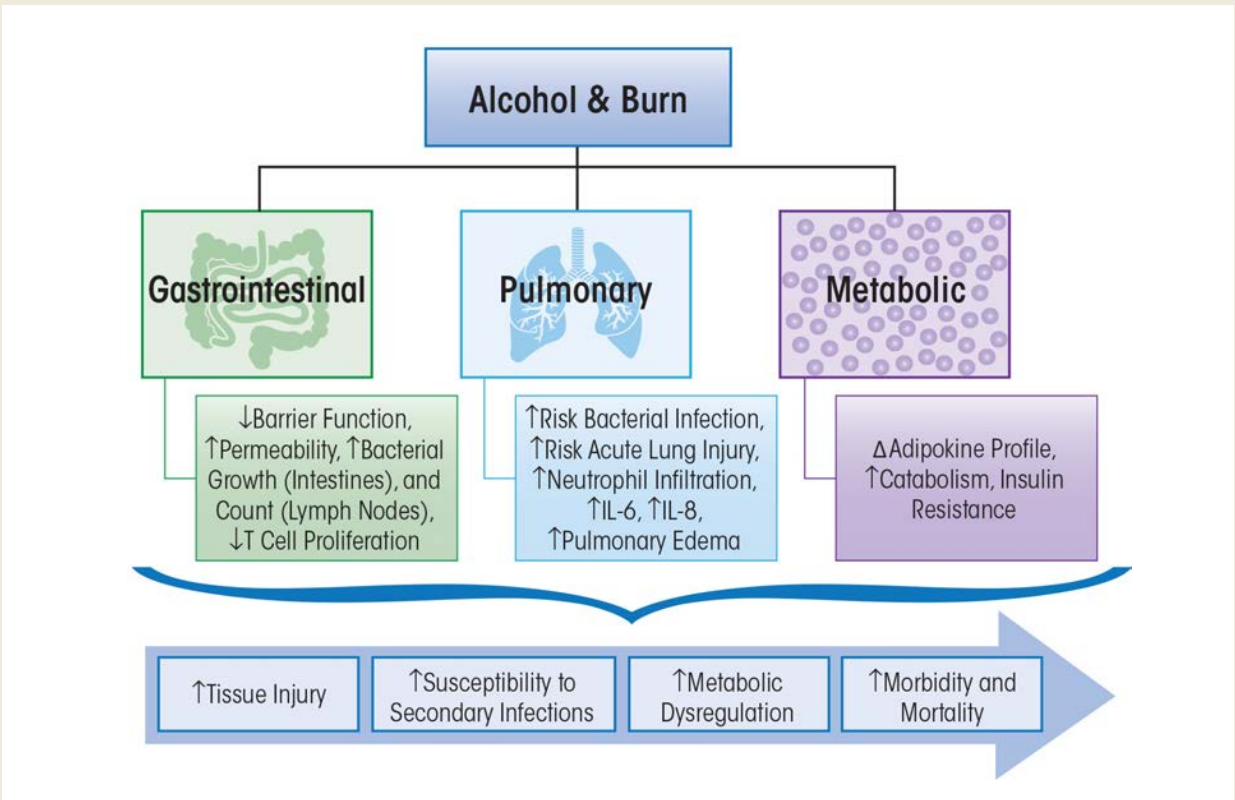

Figure 1 Salient gastrointestinal, pulmonary, and metabolic pathophysiological consequences of alcohol abuse prior to, or at the time of, burn injury. The decrease in gut barrier function leads to increased permeability and bacterial translocation that enhances the risk for bacterial infections and lung injury. Marked alterations in metabolic responses, characterized by altered adipokine profile consistent with increased insulin resistance, collectively contribute to greater morbidity and mortality post–burn injury.

exacerbating the pro-oxidative environment that has been proposed to contribute to impaired wound healing in burn patients (Herndon and Tompkins 2004). Chronic binge alcohol consumption also has been shown to promote a pro-oxidative and pro-inflammatory milieu (Rashbastep et al. 1993), and these factors may further impede wound healing in patients consuming alcohol prior to experiencing burn injury. Additional research is needed to better understand immunomodulation effects following the combined insults of alcohol and burn injury and the mechanisms underlying the more severe outcome of burn injury with alcohol abuse.

Pulmonary System

Adult respiratory distress syndrome (ARDS) is a frequent cause of death in burn patients. The lungs are one of the first organs to fail following traumatic injury (Turnage et al. 2002). Chronic and acute alcohol abuse impair pulmonary host defense to infection, thus increasing the risk of bacterial infection and acute lung injury (Boe et al. 2009; Happel and Nelson 2005). Lung injury as a result of the combination of alcohol intoxication and burn injury may be attributed to the delicate architecture of the lungs combined with other alcohol-related factors, such as bacterial and endotoxin leakage from the gut and a higher risk of contact with pathogens from the circulation and airways (Bird and Kovacs 2008; Li et al. 2007). Previous studies show that the combined insult of acute alcohol consumption and burn injury in mice leads to increased infiltration of the lungs by white blood cells, called neutrophils, and pro-inflammatory cytokine expression of IL-6 (Chen et al. 2013). Systemic and pulmonary IL-6 reflect the inflammatory state of the host and have been shown to be decreased in the absence of Toll-like receptor-4 (TLR-4) and intercellular adhesion molecule-1 (ICAM-1) (Bird et al. 2010). The role of IL-6 in lung injury has been demonstrated in studies in IL-6 knockout mice or following neutralization of IL-6, both of which result in significantly reduced lung inflammation (Chen et al. 2013). Studies also have shown that acute alcohol intoxication at the time of burn injury induces an upregulation of IL-18 production and neutrophil infiltration within the lung compartment, all leading to pulmonary edema (Li et al. 2007).

Metabolism

The post-burn period is characterized by a hypermetabolic state (Pereira and Herndon 2005) consisting of increased oxygen consumption; increased breakdown of glycogen, fats, and proteins; elevated resting energy expenditure and glucose synthesis; and reduced insulin-stimulated glucose uptake into skeletal muscle and adipose tissue (Gauglitz et al. 2009). Previous studies suggest that development of this hypermetabolic state during the post-burn period occurs as a consequence of (1) increased plasma catecholamine and corticosteroid concentrations (Jeschke et al. 2008; Williams et al. 2009; Wilmore and Aulick 1978), (2) increased systemic pro-inflammatory mediator expression, favoring processes that release energy (i.e., catabolic) over those that store energy (i.e., anabolic) (Jeschke et al. 2004), and (3) increased adipose tissue mRNA (Zhang et al. 2008) and protein (Yo et al. 2013) expression of uncoupling protein-1 (UCP-1), enhancing heat production and metabolism. Further, circulating levels of TNF-α, a known anti-insulin cytokine, are increased (Keogh et al. 1990), and the post-burn period can be described as a state of marked insulin resistance (IR) (Gauglitz et al. 2009). Insulin sensitivity has been reported to be decreased by more than 50 percent at 1-week post–burn injury in pediatric patients (Cree et al. 2007) as well as in rodent models of burn injury (Carter et al. 2004). The relevance of insulin levels to overall outcome from burn injury is supported by results from clinical studies showing that exogenous insulin therapy in pediatric burn patients decreased pro-inflammatory cytokines, increased anti-inflammatory cytokines, and increased serum concentrations of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3). Together, these changes could help to preserve organ function and better promote anabolic processes during the post-burn hypermetabolic state (Jeschke et al. 2004). Chronic alcohol consumption decreases insulin responsiveness and can alter insulin signaling through various mechanisms, including increased hepatic protein expression of the gene phosphatase and tensin homologue (PTEN), which directly inhibits insulin signaling through the phosphatidylinositol-5,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt) pathway (de la Monte et al. 2012). In addition to the negative regulation of the pathway by PTEN proteins, the enzyme protein tyrosine phosphatase dephosphorylates and decreases activity of important molecules involved in the insulin signaling cascade, potentially contributing to impaired insulin action (Gao et al. 2010; Koren and Fantus 2007). In addition, Lang and colleagues (2014) demonstrated that chronic alcohol consumption reduces Akt and AS160 phosphorylation, reduces membrane localization of glucose transporter type 4 (GLUT-4) protein, and increases serine phosphorylation at serine-307 of insulin receptor substrate-1 (IRS-1), all of which will attenuate insulin-stimulated skeletal muscle glucose uptake and other insulin-mediated anabolic effects (Lang et al. 2014). These negative effects on insulin signaling occurred in conjunction with sustained increases in pro-inflammatory cytokines TNF-α and IL-6 following chronic alcohol exposure (Lang et al. 2014). Thus, both burn injury and chronic alcohol exposure alter metabolic pathways—favoring catabolic and opposing anabolic pathways—possibly resulting in long-lasting alterations in metabolic processes. The metabolic dysregulation

following burn injury is likely to produce more severe consequences in chronic alcohol burn victims. Previous studies assessing nutritional status of alcoholic patients have been discordant, with some studies suggesting that increased alcohol consumption increases the prevalence of malnutrition in alcoholic patients (Hillers and Massey 1985), whereas other studies do not show a role for excessive, or chronic, alcohol consumption in malnutrition (Nicolas et al. 1993; Urbano-Marquez et al. 1989). A study assessing the influences of aging and chronic alcohol feeding in mice on protein synthesis demonstrated that chronic alcohol feeding decreases gastrocnemius muscle protein synthesis, which provides a mechanism for loss of lean body mass (Korzick et al. 2013; Lang et al. 2014). Decreased anabolism during the post-burn period, which itself is a state of heightened catabolic processes, could significantly impair recovery for these alcoholic patients experiencing burn injury. Further, the hypermetabolic state of the post-burn period is thought to contribute to delayed or impaired wound healing, increased susceptibility to infections, and erosion of lean body mass (Pereira and Herndon 2005). Moreover, both binge alcohol consumption (Pravdova and Fickova 2006; You and Rogers 2009) and burn injury (Venkatesh et al. 2009; Wade et al. 2013) can contribute to dysregulation of cytokines secreted by adipose tissue (i.e., adipokines). Recent studies show that mice exposed to a single alcohol binge prior to burn injury have a dramatic increase in pro-inflammatory response and a decrease in anti-inflammatory response in adipose tissue (Qin et al. 2014). The heightened pro-inflammatory response during the post-burn period would be predicted to modulate leptin levels. Thus, recovery from burn injury is likely to be severely impaired in alcoholic individuals as a result of a greater disruption in metabolic processes as well as impairment of host defense mechanisms, leading to greater morbidity and health care costs associated with the management of these patients. Therefore, further investigation is warranted to understand the modulation of the immune system by the combined effect of alcohol and burn that might result in dysregulation of adipose tissue and altered metabolism.

Alcohol and Hemorrhagic Shock

Studies from several investigators have provided evidence that traumatic injury and hemorrhagic shock produce an immediate upregulation of pro-inflammatory cytokine gene product expression (Ayala et al. 1991; Hierholzer et al. 1998). The early pro-inflammatory response is later followed by suppression of stimulated pro-inflammatory cytokine release (Angele et al. 1999; Xu et al. 1998) and alterations of both cellular and humoral immunity (Napolitano et al. 1995; Wichmann et al. 1998), leading to generalized immunosuppression, which ultimately results in an increased susceptibility to infection (Abraham 1993; Ertel et al. 1993). Along with marked alterations in hemodynamic homeostasis and neuroendocrine regulation, immunological derangements and subsequent infections are also a major cause of increased morbidity and mortality following hemorrhagic shock (Livingston and Malangoni 1988; Phelan et al. 2002).

Studies focused on the immune modulatory effects of alcohol exposure following hemorrhagic shock have demonstrated that even 24 hours after the post-hemorrhagic shock, alcohol-intoxicated animals had a marked suppression in cytokine release to an inflammatory challenge (Greiffenstein et al. 2007), affecting the ability to fight secondary infectious challenges. Conversely, findings observed at the tissue level determined that alcohol intoxication enhanced the pro-inflammatory milieu following hemorrhagic shock, priming tissues for injury. The burden of alcohol and hemorrhagic shock on specific target organ systems is discussed below and summarized in figure 2.

Gastrointestinal Tract

Hemorrhagic shock produces similar alterations in gut barrier function to those resulting from burn injury. Alcohol intoxication at the time of hemorrhagic shock further exacerbates hemorrhagic injury-induced gut permeability and leakage (Sulzer et al. 2013). Chronic alcohol consumption has been shown to disrupt intestinal barrier function and induce gut leak (Li et al. 2008; Tang et al. 2009). The combination of greater hypotension and inadequate tissue blood flow (i.e., hypoperfusion) observed in alcohol-intoxicated animals and the increased gut leak observed in alcohol-intoxicated hemorrhaged animals are speculated to contribute to increased host susceptibility to infection and tissue injury during recovery (Molina et al. 2013). Alcohol-intoxicated, hemorrhaged animals have been shown to have greater reduction in hepatic, renal, and intestinal blood flow than that observed in nonintoxicated animals (Sulzer et al. 2013). This reduction in critical organ blood flow was associated with enhanced tissue damage. An additional mechanism that could contribute to tissue injury in the alcohol-intoxicated, hemorrhaged host is the disruption of gut-associated lymphoid tissue function, which has been shown to play a role in other disease states.

Cardiovascular System

Studies using a rodent model of binge-like alcohol consumption prior to hemorrhagic shock have shown that acute alcohol intoxication decreases basal mean arterial blood pressure (MABP), exacerbates hypotension, and attenuates blood pressure recovery during fluid resuscitation (Mathis et al. 2006; Phelan et al. 2002). Following fixed-volume hemorrhage, alcohol-intoxicated animals were significantly more hypotensive throughout the

hemorrhage and resuscitation periods (Mathis et al. 2006). In response to a fixed-pressure (40 mmHg) hemorrhage, a significantly lesser amount of blood was removed from the alcohol-intoxicated animals than controls (Phelan et al. 2002). Similarly, McDonough and colleagues, using a guinea pig model of ethanol exposure prior to hemorrhagic shock (loss of 60% blood volume) and resuscitation, demonstrated that a low dose of ethanol (1 g/kg) decreases MABP and heart rate and exacerbates the metabolic effects of hemorrhagic shock, as shown by increased glucose and lactate concentrations (McDonough et al. 2002). Despite the plethora of previous studies that have examined functional cardiovascular consequence of hemorrhagic shock and hemorrhage with alcohol intoxication, few studies have examined the combined effects of alcohol, hemorrhagic shock, and immune dysfunction on the cardiovascular system. However, exacerbation of pre-existing cardiovascular disease and prolonged recovery are anticipated outcomes of the combined effects of alcohol and hemorrhagic shock, all leading to an impaired immune response.

Pulmonary System

As mentioned previously, alcohol intoxication produces significant dysregulation of the host defense mechanism during the post-injury period. Lung IL-6 and TNF-α are suppressed, while granulocyte-colony stimulating factor (GCSF) mRNA is increased in alcohol-intoxicated, hemorrhaged animals (Mathis et al. 2006; Ono et al. 2004). Moreover, isolated pleural cells and peripheral blood mononuclear cells (PBMCs) from alcohol-intoxicated, hemorrhaged animals display suppressed TNF-α, IL-1β, and IL-6 release following lipopolysaccharide stimulation (Greiffenstein et al. 2007), suggesting greater impairment of humoral immune response than that resulting from hemorrhagic shock alone. The importance of these alterations in host defense mechanisms was demonstrated in animals inoculated with *Klebsiella pneumonia* following hemorrhagic shock. These studies showed suppressed neutrophil response,

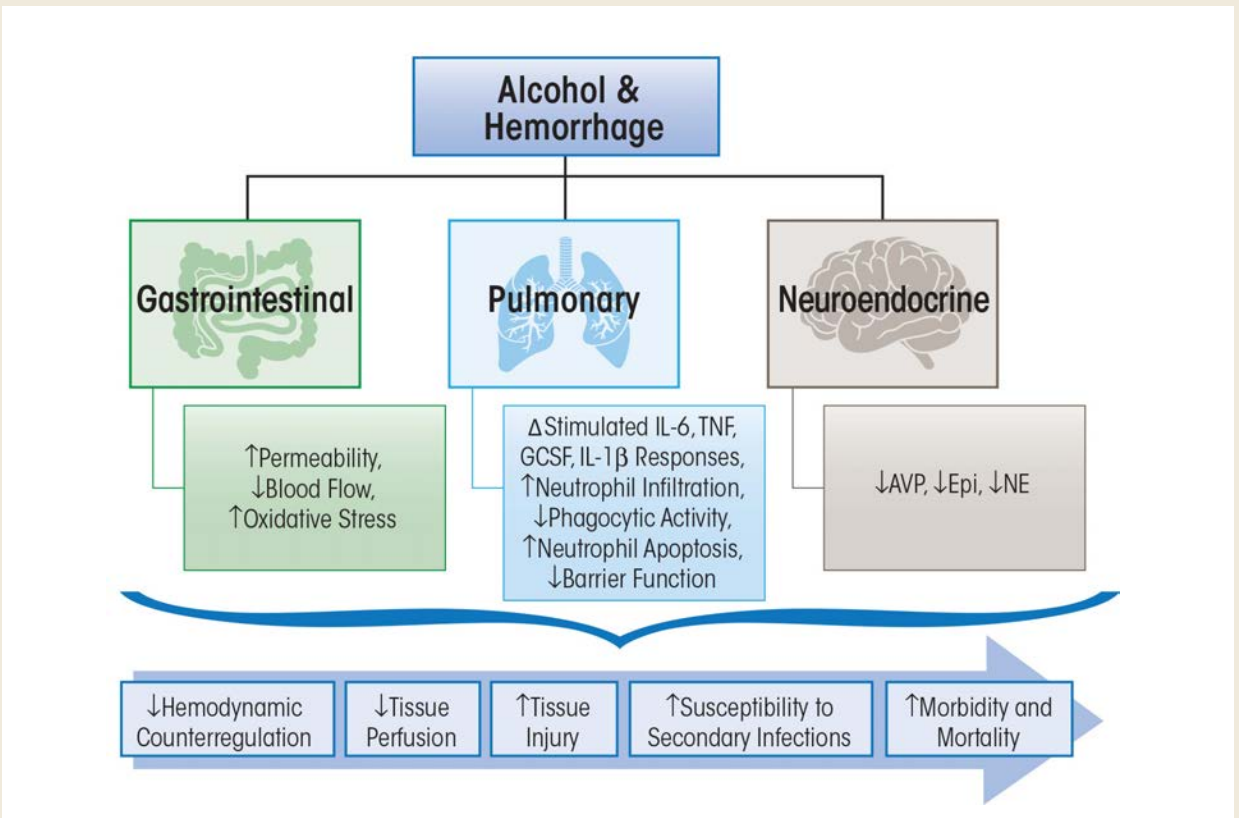

Figure 2 Salient gastrointestinal, pulmonary, and neuroendocrine pathophysiological consequences of alcohol abuse prior to, or at the time of, hemorrhagic shock. The decreased hemodynamic counterregulatory response leads to decreased tissue perfusion, accentuated oxidative stress, and enhanced tissue injury. In addition, the alcohol/hemorrhaged host shows greater susceptibility to secondary infections leading to increased morbidity and mortality during the post-injury period.

decreased phagocytic activity, and increased neutrophil apoptosis in hemorrhaged animals that were alcohol intoxicated at the time of injury (Zambell et al. 2004). This was associated with greater lung bacterial counts and prolonged elevation in TNF-α and IL-6 levels (18 h) post-infection. Furthermore, only 30 percent of alcohol-intoxicated, hemorrhaged animals survived compared with 70 percent survival of dextrose/hemorrhage animals (Zambell et al. 2004). In addition to cytokine dysregulation, alcohol impairs innate barrier functions of the lung by increasing epithelial cell permeability and altering the function of the ciliated epithelium (Elliott et al. 2007; Molina et al. 2010).

Neuroendocrine System

The pathophysiology of traumatic-hemorrhagic injury involves decreased blood volume (i.e., hypovolemia) and hypoperfusion, which results in signaling to central cardiovascular centers aimed at restoring hemodynamic stability through activation of descending autonomic neuroendocrine pathways (Molina 2005). Several mechanisms have been proposed to account for the increased hypotension and impaired hemodynamic stability observed with alcohol intoxication, with one proposed mechanism being blunted neuroendocrine activation. Studies demonstrated that acute alcohol intoxication at the time of injury results in significant attenuated release of counterregulatory hormones and potent vasoconstrictors such as arginine vasopressin (AVP), epinephrine, and norepinephrine in response to fixed-pressure hemorrhage (Phelan et al. 2002). A disruption in the neuroendocrine response with alcohol intoxication at the time of injury is associated with enhanced expression of lung and spleen TNF-α as well as suppression of circulating neutrophil function, which would be expected to enhance the risk for tissue injury (Whitaker et al. 2010). Conversely, Sato and colleagues (2013) demonstrated that alcohol aggravates hemorrhagic shock in a dose-dependent manner not by triggering an immune response but by suppressing hormonal and neurohumoral responses, thereby inhibiting hemodynamic auto-regulation and shortening the survival interval. Thus, both alcohol and hemorrhagic shock have detrimental effects on neuroendocrine responses that are likely to modulate the host immune system in addition to impacting on hemodynamic stability and recovery and accentuating tissue hypoperfusion and end-organ injury.

Alcohol and Traumatic Brain Injury

Traumatic brain injury (TBI) accounts for approximately 50 percent of all trauma-related mortality (Centers for Disease Control and Prevention 2012*b*). TBI affects multiple sectors of the population, and young males have the highest rates of hospital visits and death (Faul et al. 2010). Falls are the first leading cause of TBI, followed by motor vehicle accidents and unintentional trauma sustained during sports activities such as football or boxing. TBI can be categorized as mild, moderate, or severe, and the majority of TBIs sustained in the United States are in the mild category (Centers for Disease Control and Prevention 2012*b*). In addition to the physical dysfunction caused by injury, TBI patients frequently experience lingering psychological symptoms, such as heightened anxiety, depression, sleep disturbances, and pain hypersensitivity (Whyte et al. 1996). These symptoms have been implicated in increased alcohol intake following TBI in humans (Adams et al. 2012). Furthermore, it is well accepted that alcohol consumption increases the risks of sustaining a TBI (Corrigan 1995; Hurst et al. 1994). Nevertheless, a comprehensive understanding of the influences of alcohol on TBI-induced inflammation, recovery from injury, and long-term damage currently is limited and is summarized in the following section (see figure 3).

Neuroinflammation

The pathophysiology of TBI involves a primary mechanical injury followed by a secondary tissue injury resulting from neuroinflammation (Werner and Engelhard 2007). A large percentage of TBI victims show signs of further deterioration following the event (Sauaia et al. 1995). This suggests the induction of a secondary brain injury and immune activation as the key cascades contributing to the pathophysiological processes of the secondary damage (Cederberg and Siesjo 2010). After TBI, a series of events occurs, including the activation of resident immune cells such as astrocytes and microglia, release of pro-inflammatory cytokines and chemokines, upregulation of endothelial adhesion molecules, and recruitment and activation of blood-derived leukocytes across the disrupted blood brain barrier (Feuerstein et al. 1998; Morganti-Kossmann et al. 2001; Ransohoff 2002). An increase in the levels of TNF-α in the serum or cerebrospinal fluid in victims of TBI also has been detected in rodents following closed head injury (Goodman et al. 1990; Ross et al. 1994; Shohami et al. 1994). IL-1β is released after TBI (Fan et al. 1995) and induces nuclear factor-kappa B (NF-κ B), a key transcription factor that regulates the expression of genes encoding cytokines, as well as inducible NO synthase (iNOS), and cyclooxygenase-2 (COX-2) (Blanco and Guerri 2007; Woodroofe et al. 1991; Ziebell and Morganti-Kossmann 2010). Following the rise of early cytokines, the release of IL-6 is associated with increased acute-phase proteins, as well as blood–brain barrier disruption (Kossmann et al. 1995; Shohami et al. 1994; Woodcock and Morganti-Kossmann 2013) and sustained elevation of chemokines such as chemokine (C-C motif) ligand-2 (CCL-2) in the cerebrospinal fluid for as long as 10 days post-injury (Semple et al. 2010). Although early

cytokine release is essential in mediating the reparative processes after injury (Ziebell and Morganti-Kossmann 2010), sustained elevation of pro-inflammatory mediators has been increasingly recognized to play a role in neuropathological changes associated with long-term degenerative diseases (Fan et al. 1995; Lyman et al. 2014). Accordingly, the additional risks of alcohol as a factor contributing to the alterations of TBI-induced neuroinflammatory processes may affect the overall recovery.

Alcohol exerts a profound impact on neuroinflammation. Although there are some conflicting reports in the literature about the role of alcohol on recovery, the major findings are summarized here. Some animal studies suggest that acute alcohol administration prior to TBI leads to an early reduction in the levels of pro-inflammatory cytokines and chemokines in the injured cortex, hippocampus, and hypothalamus, as well as in the serum shortly after TBI (Goodman et al. 2013; Gottesfeld et al. 2002). Recent studies also have confirmed that acute alcohol intoxication at the time of TBI does not exacerbate the expression of pro-inflammatory cytokines and chemokines at 6 hours post-injury. However, results obtained at a later time point (24 hours) show a sustained mRNA expression of IL-1β, TNF-α, IL-6, and CCL-2 following a lateral fluid percussion injury in rodents that were alcohol-intoxicated at the time of TBI (Teng and Molina 2014). Overall, some preclinical studies suggest that acute alcohol treatment prior to TBI may lead to a suppressed release of pro-inflammatory mediators during the early phase post-injury. Thus, the temporal pattern of neuroinflammatory responses and the impact of alcohol intoxication on neuroinflammatory responses are factors to consider when drawing conclusions on the role of alcohol in modulating the outcome from TBIs.

Because the literature surrounding the relationship between acute alcohol intoxication and response to trauma is conflicting, it is important to consider the pattern of alcohol abuse and the

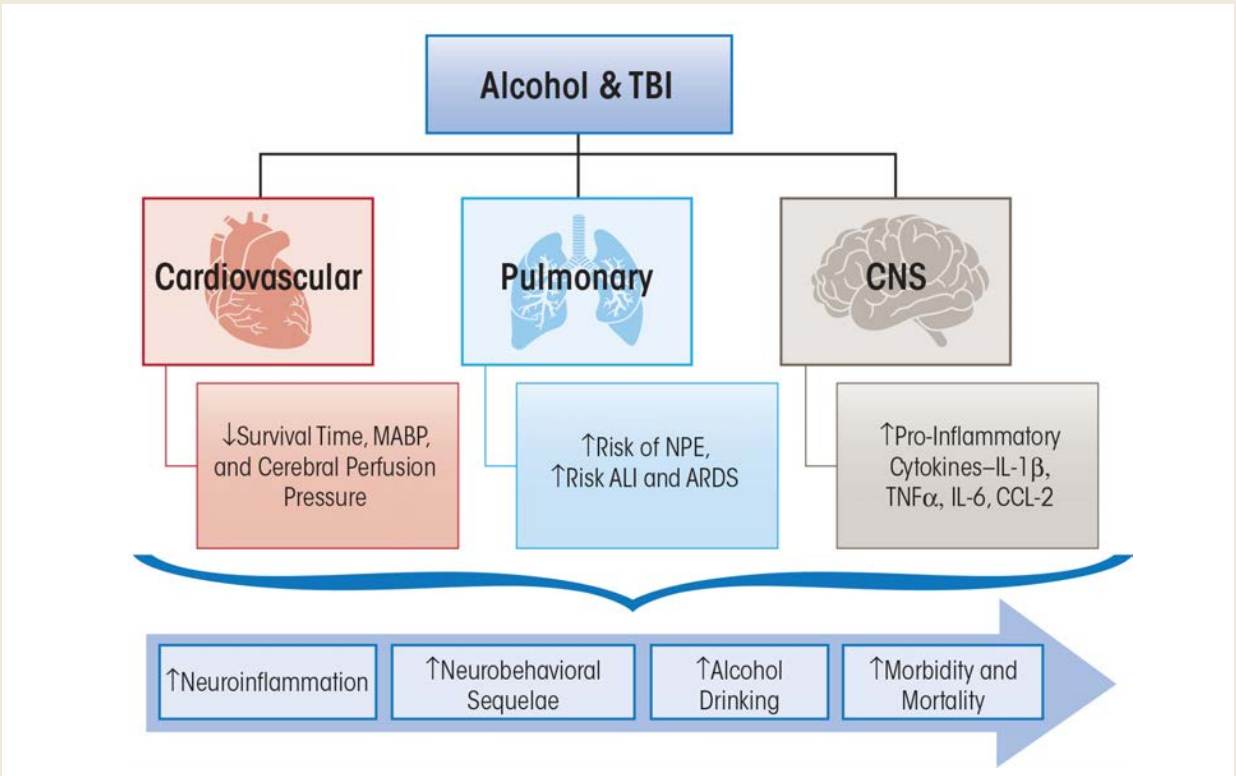

Figure 3 Salient cardiovascular, pulmonary, and central nervous system pathophysiological consequences of alcohol abuse prior to, or at the time of, traumatic brain injury (TBI). The disruption in hemodynamic homeostasis resulting from TBI contributes to decreased cerebral perfusion pressure. The lung is affected through neurogenic mechanisms leading to neuropulmonary edema (NPE) and associated risk for acute lung injury (ALI) and adult respiratory distress syndrome (ARDS). In the brain (CNS), alcohol accentuates neuroinflammation, which is associated with neurobehavioral dysfunction that can potentially promote alcohol drinking. Together, these pathophysiological consequences increase morbidity and mortality from TBI.

model used in different studies. In general, reports in the literature indicate that chronic alcohol exposure produces immune activation in the brain, inducing an enhanced pro-inflammatory state, as evidenced by the presence of CCL-2 and microglial activation in postmortem brains of human alcoholics (He and Crews 2008). Animal studies show that chronic, intermittent binge alcohol administration to rodents results in increased microglial activation and inflammatory cytokine expression in the cortex and hippocampus (Zhao et al. 2013). In addition, Crews and colleagues (2004) have found that chronic alcohol treatment induces expression of inflammatory cytokines such as TNF-α, which further activates resident glial cells to secrete additional pro-inflammatory cytokines and chemokines, resulting in an increased immune activation in the brain. The overall pro-inflammatory effects of alcohol also have been shown by Guerri and colleagues (2007) who reported alcohol-mediated stimulation of TLR-4 and IL-1 receptor signaling pathways, including extracellular regulated-kinase 1/2 (ERK1/2), stress-activated protein kinase/c-Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinase (MAPK), as well as the expression of NF-kB, activator protein-1 (AP-1), iNOS, and COX-2 in cultured glial cells (Alfonso-Loeches et al. 2010; Fernandez-Lizarbe et al. 2009). The role of TLR4 has been identified in studies where 5 months of chronic alcohol administration increased glial activation and levels of caspase-3, iNOS, COX-2, and cytokines (IL-1β, TNF-α, and IL-6) in the cerebral cortex of wild-type mice but not in the TLR-4–deficient mice (Alfonso-Loeches et al. 2010). Another mediator of alcohol-mediated neuroinflammation is high-mobility group protein B1 (HMGB1), which has been reported to be increased along with TLR-2, TLR-3, and TLR-4 in postmortem brains of human alcoholics (Alfonso-Loeches et al. 2010). Despite a substantial amount of evidence showing increased neuroinflammatory responses to chronic alcohol exposure, there have not been sufficient preclinical studies performed to determine the combined effect of chronic alcohol consumption and TBI on neuroimmune activation. Because both TBI and alcohol can induce inflammation in the brain, we speculate that the combination of the two events would further accentuate neuroinflammation.

Alcohol combined with traumatic injury can significantly affect morbidity and mortality through disruption in host immune responses.

Retrospective studies have revealed that outside of the central nervous system, peripheral organ damage can contribute to the increased mortality rate among TBI patients as a result of cardiovascular, pulmonary, and endocrine dysfunction (Gennarelli et al. 1989; Shavelle et al. 2001). More specifically, TBI patients have an increased incidence of ALI, pulmonary infection, neuroendocrine alterations, and cardiovascular dysfunction during the post-injury period (Vermeij et al. 2013). Although the combined effects of alcohol and TBI and the role of local or systemic immune responses in peripheral organs are understudied, the current knowledge is summarized below (figure 3).

Pulmonary System

ALI, one of the most common nonneurologic complications following TBI, results from acute pulmonary edema and inflammation and can lead to ARDS (Holland et al. 2003; Johnson and Matthay 2010). ALI is characterized by hypoxemia, loss of lung compliance, and bilateral chest infiltrates (Dushianthan et al. 2011). Development of ALI post-TBI has been associated with increased inpatient mortality following injury and worse long-term neurologic outcome in survivors of TBI (Bratton and Davis 1997; Holland et al. 2003). Post-TBI medical interventions including induced systemic hypertension and mechanical ventilation can result in nonneurogenic ALI (Contant et al. 2001; Lou et al. 2013). Development of neurogenic pulmonary edema (NPE) occurs minutes to hours following TBI and typically resolves within days (Bratton and Davis 1997). The possible underlying factors in NPE are the severity of injury leading to increased intracranial pressure and the subsequent increased circulating catecholamines (Demling and Riessen 1990). TBI also is associated with greater incidence of pulmonary infections than that seen following major surgeries, burn injury, and polytrauma (Dziedzic et al. 2004). Clinical reports indicate that over 40 percent of TBI patients with artificial ventilation develop pneumonia and are four times more likely to die from pneumonia (Harrison-Felix et al. 2006). The increased risk of developing pneumonia post-TBI is potentially attributed in part to a systemic immune response syndrome (SIRS) characterized by increased circulating pro-inflammatory cytokines (TNF-α and IL-6) (Keel and Trentz 2005; Kossmann et al. 1995).

The combined impact of alcohol and TBI on pulmonary infections has been minimally investigated. Although, epidemiological studies have shown that in trauma patients, chronic alcohol abuse can independently increase the risk of ALI and ARDS two- to four-fold (Guidot and Hart 2005). In a prospective study of traumatic injury patients with evidence of acute alcohol intoxication or chronic alcohol abuse, chronic alcohol was associated with increased incidence of pneumonia or respiratory failure as a result of its immunosuppressive effects. However, no significant increase in incidence of pneumonia or respiratory failure and

mortality was observed in patients with acute alcohol intoxication with BAC above 100mg/dL (De Guise et al. 2009; Jurkovich et al. 1993). The importance of length and amount of pre-existing alcohol intake and TBI severity may be the key factors in determining a patient's risk for pneumonia. Taken together, the potential effects of chronic alcohol abuse and TBI could potentiate and further increase immunosuppression or immune dysfunction, thus leading to greater susceptibility for pneumonia, ARDS, and ultimately death.

Neuroendocrine System

TBI can lead to a variety of neuroendocrine abnormalities, such as gonadotropin deficiency, growth hormone deficiency, corticotrophin deficiency, and vasopressin alterations (Behan and Agha 2007; Powner and Boccalandro 2008). As a result of the mechanical compression to the pituitary gland or disruption of the pituitary stalk, hypopituitarism can occur and corticotrophin insufficiency is commonly observed after TBI (Agha et al. 2004; Cohan et al. 2005). Excessive alcohol use also has been reported to be associated with neuroendocrine dysfunction, notably in the form of altered regulation of hypothalamic–pituitary–adrenal axis (HPA), resulting in a decreased corticotrophin release (Behan and Agha 2007; Helms et al. 2014). Therefore, it is possible that the combination of alcohol and TBI-induced HPA dysfunction can lead to a dampened cortisol release, which may have an impact on the immune system. Interestingly, a hyperadrenergic state marked by elevated levels of catecholamines can occur after TBI, and alcohol intoxication at the time of TBI has been shown to blunt the sympatho-adrenal activation in a dose-dependent manner (Woolf et al. 1990). Vasopressin has been suggested to play a role in blood brain barrier disruption, edema formation, and the production of pro-inflammatory mediators after TBI (Szmydynger-Chodobska et al. 2010). Vasopressin abnormalities leading to diabetes insipidus or the syndrome of inappropriate anti-diuretic hormone (SIADH) frequently are observed after TBI (Behan and Agha 2007), and acute alcohol intoxication is known to alter AVP release (Taivainen et al. 1995). Whether alcohol intoxication at the time of TBI or during the recovery period from TBI further dysregulates these neuroendocrine mechanisms remains to be examined.

Cardiovascular System

Cardiovascular complications including slow heart rate (i.e., bradycardia), hypotension, electrocardiographic changes, arrhythmias, and increased circulating cardiac enzymes have been reported following TBI (Bourdages et al. 2010; Wittebole et al. 2005). Chronic alcohol abuse alone can lead to alcoholic cardiomyopathy and potentially heart failure (Skotzko et al. 2009), and the underlying etiology has been reviewed (Lang et al. 2005). Several studies by Zink and colleagues (1998*a*,*b*, 2006) focused on the combined effects of acute alcohol intoxication on hemorrhagic shock and TBI in swine, showing decreased survival time, lowered MABP, and reduced cerebral perfusion pressure, which may worsen secondary brain injury. These studies did not investigate alterations in immune function or expression and levels of immune modulators or their actions on cardiovascular function. Overall, the post-TBI cardiovascular complications, including vascular function, have been understudied in both clinical and experimental models of TBI. More specifically, the combined impact of alcohol, TBI, and immune alterations on cardiovascular dysfunction and disease progression has not been examined. A possible prediction is that chronic alcohol-induced immunosuppression would worsen post-TBI cardiovascular complications; and in chronic alcoholics, dilated cardiomyopathy may compound TBI-related cardiovascular complications increasing morbidity and mortality.

Summary

The deleterious effects of alcohol on the immune system in three traumatic injuries are discussed in this review and are summarized in figures 1, 2, and 3. It is evident that, independently, acute or chronic alcohol consumption and traumatic injury negatively modulate the immune system, and the end result is an uncontrolled release of inflammatory mediators. The most important message of this review is the accumulation of evidence that alcohol combined with traumatic injury can significantly affect morbidity and mortality through disruption in host immune responses. Following burn injury, for instance, the risk for infection is greatly increased because of increased gut permeability and increased pro-inflammatory cytokine expression in the lungs (figure 1). Alcohol use following hemorrhage can increase inflammation and oxidative stress in the gut while decreasing lung barrier function and subsequently increasing susceptibility to infection (figure 2). In the central nervous system, alcohol use following TBI can increase neuroinflammation and prolong the recovery period (figure 3). Overall this information is important, because it provides a wealth of evidence that alcohol combined with trauma is a dramatic and preventable cause of increased morbidity and mortality following injury. Mechanistically, two common pro-inflammatory cytokines that are consistently upregulated in all burn injury, hemorrhagic shock, and TBI are TNF-α and IL-6. A fuller understanding of their temporal pattern of expression and downstream effects requires further investigation. Although the studies described in this review have generated important information on the impact of alcohol combined with different types of traumatic injury, and the resultant adverse effects on the immune system, further preclinical

and clinical studies to dissect the complex cascade of immunomodulation following injury are necessary. Specifically, further investigation is warranted to determine the underlying mechanisms involved in immune modulation by acute or chronic alcohol intake and the effects on (1) metabolism and the cardiovascular system following burn, (2) the neuroendocrine system following hemorrhagic shock, and (3) neuroinflammation and the neuroendocrine system following traumatic injury. The responses of the immune system to these inflammatory stimuli are variable and appear to be dependent on the severity of the injury, comorbidities, and the level of alcohol intoxication. Thus, it is necessary to systemically address these variables for translational research to identify potential therapeutic strategies. Furthermore, therapeutic targets for immunomodulation and attenuation of tissue injury in intoxicated and injured patients are likely to reduce morbidity and mortality and improve post-injury quality of life among these patients.

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Neuroplasticity and Predictors of Alcohol Recovery

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Chronic alcohol-related neuroadaptations in key neural circuits of emotional and cognitive control play a critical role in the development of, and recovery from, alcoholism. Converging evidence in the neurobiological literature indicates that neuroplastic changes in the prefrontal–striatal–limbic circuit, which governs emotion regulation and decisionmaking and controls physiological responses in the autonomic nervous system and hypothalamic–pituitary–adrenal axis system, contribute to chronic alcoholism and also are significant predictors of relapse and recovery. This paper reviews recent evidence on the neuroplasticity associated with alcoholism in humans, including acute and chronic effects, and how these neurobiological adaptations contribute to alcohol recovery, along with the discussion of relevant clinical implications and future research directions.

Key words: Alcohol use, abuse, and dependence; alcoholism; alcohol effects and consequences; alcohol-related neuroadaptations; neurobiology; brain; neuroplasticity; prefrontal-striatal-limbic circuit; autonomic nervous system; hypothalamic-pituitary-adrenal axis system; recovery; relapse

Recovery from alcoholism is a complex and long-term process with high relapse rates. Therefore, understanding why people relapse has been critically important to improving treatment outcomes. To that end, researchers are looking for clinical and biological markers that predict relapse after treatment and to use those risk factors to develop effective treatments to reduce relapse rates. One promising research area is examining how alcohol changes structure and function in the brain, affecting what neuroscience calls neuroplasticity and causing neuroadaptations that can affect the brain's reward and decision-making centers and, in turn, affect relapse and recovery.

During recovery, individuals with alcohol use disorder (AUD) psychologically and physiologically recuperate from the deleterious effects of alcohol exposure by achieving complete abstinence or low-level, nonhazardous alcohol intake. The National Epidemiologic Survey of Alcohol-Related Conditions (NESARC) conducted 43,093 in-person interviews with a national sample of adults and found that 4,422 subjects, at some point prior to the past year, met the criteria for alcohol dependence, as defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM–IV). According to the survey, during the preceding year, of those 4,422 alcohol-dependent people, 35.9 percent achieved either low-risk drinking (17.7 percent) or abstinence (18.2 percent) (Dawson et al. 2005). The study also noted that recovery rates tend to be even lower in clinical samples of people with severe dependence and for people with lifetime dependence or at high risk of relapse (Dawson et al. 2005). Additionally, the risk of relapse after treatment for AUD increases if people have concurrent conditions, such as anxiety or stress sensitivity (Kushner et al. 2005; Sinha et al. 2011).

In an effort to identify clinical and biological markers that predict relapse risk, researchers have looked toward the brain and alcohol-related changes in the brain that might make it more difficult for people with AUD to recover successfully. In particular, recent research has capitalized on advances in neuroimaging techniques to examine neuroplastic changes that may increase vulnerability to alcoholism and alcohol relapse (Buhler and Mann 2011). In fact, evidence suggests that chronic, heavy alcohol consumption is related to neuronal changes that target critical central nervous system (CNS) functions governing homeostasis, emotion regulation, and decisionmaking. These changes, in turn, may make it significantly more challenging for people to stop drinking and may result in various comorbid, psychological,

and physiological symptoms (Bechara 2005; Breese et al. 2011). For instance, when people with AUD are abstinent, altered neural circuits of stress and reward modulation make them highly sensitive to stress and increase alcohol craving and other withdrawal symptoms, including anxiety, negative emotion, autonomic nervous system (ANS) disruption, fatigue, and sleep problems (Breese et al. 2011; Seo and Sinha 2014).

These chronic alcohol-related neuronal changes and their co-occurring symptoms, such as stress, may serve as markers of alcohol relapse and long-term recovery but are not currently addressed in most AUD treatment programs. Already there is evidence that people who maintain long-term abstinence show functional differences in resting-state brain synchrony relative to those with short-term abstinence (Camchong et al. 2013).

This paper reviews the evidence for neuronal changes associated with alcoholism in humans, including those resulting from acute and chronic effects of alcohol, and how these changes contribute to alcohol relapse. To help understand alcohol recovery in a clinical research setting, the review will specifically focus on neuroplastic changes associated with alcohol relapse immediately following treatment. This paper also reviews the effects of stress on alcohol-related neuroplasticity and alcohol recovery, along with relevant clinical implications and future research directions. Elucidating the link between neuroplastic changes and alcohol recovery will contribute to our understanding of complex alcohol-related symptomatology and provide insights into the development of effective treatments to improve recovery from alcoholism.

Neuroplastic Changes in the PSL Circuit

Neuroplasticity refers to changes in the nervous system that occur in response to various stimuli or experiences and include structural and functional re-organization (Sale et al. 2014). These neuroplastic changes can be acute or take place over time (Sale et al. 2014) and can either be positive or negative, depending on the experience (Vance and Wright 2009). Neuroplastic changes in response to alcohol or other addictive substances are most commonly regarded as negative neuroplasticity associated with suboptimal functioning and maladaptive behaviors (Kalivas and O'Brien 2008). Addiction researchers frequently use the term "neuroadaptation" when referring to alcohol- or drug-related neuroplastic changes in the CNS (Breese et al. 2011; Cohen 2003; Shaham and Hope 2005). Thus, the addiction neuroscience literature uses the concepts of neuroadaptation and neuroplasticity interchangeably.

In studies of alcoholism, substantial evidence indicates short-term and long-term pharmacological effects of alcohol on the nervous system and related neurophysiological dysfunction (Seo and Sinha 2014). Specifically, research has well documented acute and chronic alcohol-related neuroadaptations in the prefrontal–striatal–limbic (PSL) circuit, which helps modulate motivation and emotion (Buhler and Mann 2011). The circuit consists of the striatal-limbic system, which is involved in the brain's reward system in the striatum, and its stress system, in the amygdala; and the prefrontal regulatory region, which includes the medial prefrontal cortex (PFC), the anterior cingulate cortex (ACC), the orbitofrontal cortex (OFC), and the dorsolateral PFC. As a whole, the PSL circuit plays a pivotal role in modulating reward, stress, and decision-making throughout the course of alcoholism, including the disorder's initial development, alcohol dependence, uncontrollable alcohol seeking, and continued alcohol relapse despite its negative consequences (Seo and Sinha 2014).

One way this circuit could interact with alcohol to influence these phases of alcoholism is through a part of the PSL circuit called the ventromedial prefrontal cortex (VmPFC), a brain region critical for emotional and behavioral control and that regulates the ANS and hypothalamic–pituitary–adrenal (HPA) axis systems (Radley 2006). If repeated alcohol use disturbs the VmPFC, it could disrupt the regulation and homeostasis of ANS and HPA axis system functioning, which can result in high physiological and emotional arousal, and, in turn, is associated with high alcohol craving (Sinha et al. 2009). Continued chronic alcohol-related changes in the PSL circuit could place individuals in a neurobiologically vulnerable state, substantially compromising their ability to control the urge to drink heavily and increasing the risk that they will resume drinking after a period of abstinence. For this reason, researchers have suggested that maintaining an intact PSL circuit is critical to a person's ability to overcome alcohol seeking and relapse urge (Koob 2009; Seo and Sinha 2014; Sinha 2008). Thus, understanding acute and chronic neuroadaptive patterns in the course of alcohol illness, especially in the PSL circuit, may provide insights into alcohol-related clinical symptoms, emotional and behavioral changes, and the potential impact of these patterns on alcohol recovery.

Acute Effects of Alcohol on Brain Response

Alcohol has clear and immediate pharmacological effects on the brain (see for example, Wallner and Olsen 2008). Specifically, neuroimaging studies of acute alcohol consumption in healthy social drinkers find specific effects on emotional processing and modulation (Gilman et al. 2008), cognitive disruption (Soderlund et al. 2007), and decisionmaking (Gilman et al. 2012).

In relation to emotional processing and modulation, several functional magnetic resonance imaging (fMRI) studies report acute effects of alcohol on reducing anxious and negative

emotion and increasing alcohol craving by modulating limbic-striatal activity. In one study (Gilman et al. 2008), researchers administered an intoxicating dose of alcohol to healthy individuals via intravenous injection and found they had reduced limbic response to fearful faces and increased striatal activity. In another study (Sripada et al. 2011), researchers found decreased activity in the amygdala when 12 healthy but heavy social drinkers ingested alcohol and then viewed socioemotional stimuli, including fearful/angry faces. Another study (Gorka et al. 2013), using the same study sample and design, found that drinking alcohol reduced the connectivity between the amygdala and orbitofrontal cortex, suggesting that the regulatory part of the brain is interacting less with the amygdala during the processing of socioemotional stimuli under the influence of alcohol. When a different group of healthy but heavy social drinkers received an alcohol taste cue, researchers saw increased activity in the VmPFC, the ACC, and the ventral striatum (Filbey et al. 2008). Consistent with this, researchers saw enhanced activity in regions of the ventral and dorsal striatum in healthy male social drinkers asked to imagine an alcohol cue–related situation, with a significant correlation between alcohol craving and activity in these regions (Seo et al. 2011).

Several fMRI studies also have reported an influence of alcohol on cognitive function and decisionmaking. Alcohol consumption in healthy individuals resulted in impaired episodic memory encoding, which, in turn, was associated with reduced activity in the lateral PFC (Soderlund et al. 2007). In addition, during a decision-making task, acute alcohol administration via intravenous injection increased risk-taking behaviors, increased striatal reactivity to risk choices, and blunted brain response to emotional feedback related to both winning and losing (Gilman et al. 2012).

Taken together, neuroimaging studies demonstrate the significant influence of alcohol in healthy individuals via alterations in the PSL circuit, including reduced limbic response to negative emotional stimuli (Gilman et al. 2008; Sripada et al. 2011), enhanced striatal response to rewarding stimuli (Filbey et al. 2008; Gilman et al. 2008; Seo et al. 2011) and to risky decision-making (Gilman et al. 2012), and impaired episodic memory functioning (Soderlund et al. 2007). These studies clearly point to the PSL circuit as a critical early target of alcohol effects and its potential, deleterious impact on neuroplasticity with chronic alcohol abuse.

Neuroadaptations, Chronic Alcoholism, and Recovery

Not surprisingly, just as acute alcohol consumption affects the brain, so does chronic, heavy alcohol consumption. In fact, studies consistently report alcohol-related neuroadaptive changes in the PSL circuit, along with related allostatic changes in physiological functions, including ANS and HPA axis systems (Breese et al. 2011; Seo and Sinha 2014). The brain regions affected include the reward system, the stress system, and the prefrontal regulatory system (Seo and Sinha 2014).

Reward System Dysfunction and Alcohol Recovery

Several lines of research link changes in the striatum and, therefore, the brain's reward system to chronic alcohol use:

- Blunted dopamine release and other types of dopamine dysfunction in the striatum may be a biomarker indicating increased vulnerability to alcohol and other substance use (Trifilieff and Martinez 2014);
- Chronic alcohol abuse and exposure result in alterations in reward brain regions, such as the ventral striatum, leading to disrupted dopamine transmission and striatal activity (Martinez et al. 2005; Seo and Sinha 2014; Volkow et al. 2002).
- Detoxified AUD patients show signs of altered reward responses, such that enhanced ventral striatal activity is more biased toward alcohol cues than other reward cues, such as money (Wrase et al. 2007); and
- People with AUD had reduced levels of dopamine D2 receptors in their frontal-striatal regions compared with control subjects (Volkow and Fowler 2000).

Researchers have suggested that repeated alcohol use gradually enhances incentive salience and craving response toward alcoholic beverages by altering the reward pathways and triggering more alcohol craving and drug seeking (Breese et al. 2011; Robinson and Berridge 1993). Altered reward-system function, in turn, could further aggravate a lack of control over the reward response and intensify alcohol craving and the urge to drink alcohol, both of which are associated with increased vulnerability to alcohol relapse (Breese et al. 2011; Sinha 2008). Several lines of research support this theory, reporting significant associations between altered striatal response and alcohol relapse:

- Decreased levels of striatal dopamine D2 receptor persisted in AUD patients and did not recover up to 4 months after alcohol detoxification (Volkow et al. 2002).
- Patients who relapsed within 3 months after discharge had lower levels of dopamine during detoxification than patients who did not relapse, according to a study that measured dopamine in 21 AUD inpatients using [^{123}I] iodobenzamide (IBZM) single-photon emission computerized tomography (SPECT) (Guardia et al. 2000).
- AUD patients who relapsed within 3 months of becoming abstinent

showed increased activity in part of the striatum, called the ventral putamen, when viewing visual alcohol cues during the early weeks of abstinence (at least 1 week after detoxification) (Braus et al. 2001).

- On the other hand, recently detoxified (1 to 3 weeks) alcoholic patients with a blunted striatal response to positive emotional pictures relative to neutral pictures had a greater number of drinking days and a higher amount of alcohol consumed during the 6-month followup (Heinz et al. 2007).

These studies suggest that striatal reward system function plays a key role in the development of alcoholism and continues to influence the course of alcoholism by affecting alcohol recovery. Continued alcohol use seems to sensitize striatal reward function and increase incentive salience toward alcohol stimuli. In AUD patients, this altered striatal system may further intensify craving responses and trigger withdrawal symptoms during alcohol-free periods, increasing risk for relapse (Vanderschuren and Pierce 2010).

Neuroadaptations in the Neural Circuit of Stress Modulation

As excessive alcohol use continues, alterations in the reward system could result in allostatic changes in other brain regions closely connected with the striatum, including the limbic regions and the PFC (Breese et al. 2011; Koob and Volkow 2010). In particular, alterations in the stress system may play a crucial role in the well-known comorbid symptoms associated with alcohol dependence, including aversive emotional states such as anxiety, negative mood, high stress sensitivity, and stress-induced alcohol craving (for example, see Sinha et al. 2009).

Stress is a critical factor in increasing alcohol craving and compulsive alcohol consumption (Breese et al. 2005; Koob 2009), as evidenced by both preclinical and clinical studies, including overconsumption of alcohol in male mice with prenatal stress (Campbell et al. 2009), early trauma associated with greater alcohol use and alcohol craving (Schumache et al. 2006), and increased alcohol use after the 9/11 terrorist attacks among New York City residents (Vlahov et al. 2006). Individuals suffering from chronic alcoholism frequently report high stress sensitivity and stress-triggered intense craving (Fox et al. 2007; Sinha et al. 2009). And stress sensitivity plays a crucial role in increased alcohol craving to alleviate aversive emotions or stimuli (Gilpin and Koob 2008)—known as "negatively reinforced craving"—which becomes a main driving force for drinking as excessive alcohol use continues (Koob 2009; Koob et al. 2004; Sinha 2008).

Stress is a critical factor in increasing alcohol craving and compulsive alcohol consumption.

The brain's stress response involves activation of the ANS and HPA axis systems to promote regulation of physiological arousal and also facilitate adaptive coping (Sinha 2008). Chronic alcoholism is associated with impaired autonomic regulation characterized by high basal heart rate, reduced heart rate variability, and increased blood pressure (Quintana et al. 2013; Sinha et al. 2009; Stormark et al. 1998; Thayer et al. 2006). Further, upregulated HPA axis function, including elevated levels of basal cortisol and adreno-corticotrophic hormone (ACTH), has been frequently found in people with AUD (Breese et al. 2011; Sinha 2008; Sinha et al. 2009). Consistent with this, alcoholics who continue to drink, and those experiencing withdrawal symptoms, have increased levels of basal stress hormones, including cortisol, norepinephrine, and corticotropin-releasing factor (CRF) (for review, see Breese et al. 2011). In addition, a study of 93 treatment-engaged, 1-month-abstinent AUD patients found strong associations between alcohol relapse and HPA axis system function. In this study, greater morning adrenal sensitivity indexed by the cortisol-to-ACTH ratio significantly predicted a shorter time to future initial relapse as well as heavy-drinking relapse (Sinha et al. 2011), indicating a significant role of chronic alcohol-related stress pathology in alcohol recovery.

In terms of brain regions involved, researchers postulate that neuroadaptations in the amygdala may influence negatively reinforced craving and alcohol seeking (Koob 2009; Koob et al. 2004; Sinha 2008). The amygdala is involved in stress-induced physiological responses via modulation of CRF and norepinephrine pathways, which are well known for their contribution to negative reinforcement aspects of addiction (Koob 1999, 2009). Research with AUD patients abstinent for 1 week shows a potential role of altered amygdala functioning in alcohol recovery. In this study, patients who relapsed had reduced amygdala volume compared with patients who did not relapse, and the reduction of the amygdala volume was significantly associated with alcohol craving and the amount of follow-up alcohol drinking (Wrase et al. 2008). Consistent with these data, preclinical studies report associations between altered response in the extended amygdala and stress-primed drug reinstatement (for review, see Kalivas and McFarland 2003).

During stress, ANS and HPA axis function are under the regulatory control of the VmPFC (Figueiredo et al. 2003; Radley 2006). Preclinical studies demonstrate decreased HPA axis response to stress following VmPFC lesions (Radley 2006) and find that the VmPFC maintains stress-related inhibitory control over HPA axis arousal (Figueiredo et al. 2003). In addition, a meta-analysis of

studies in humans reported significant associations between brain activity in the VmPFC and amygdala and ANS function indexed by heart rate variability (Thayer et al. 2012). Given that chronic alcoholism is associated with HPA axis and ANS system dysfunctions, as discussed earlier, these findings on the VmPFC regulation over stress-related HPA axis and ANS arousal suggest that individuals with chronic alcoholism may have underlying VmPFC dysfunction in response to stress. Consistent with this hypothesis, a recent fMRI study (Seo et al. 2013) found lowered activity in the stress modulatory regions involving VmPFC/ACC during stress exposure in 30 AUD patients engaged in inpatient treatment and abstinent for 4 weeks, compared with 30 matched healthy control subjects (figure 1A). Interestingly, the researchers observed an opposite pattern when the subjects were relaxed: AUD patients showed hyperactive VmPFC/ACC compared with control subjects (figure 1A). More importantly, to prospectively assess relapse and early recovery, these researchers followed the same 30 AUD patients, plus 15 others, after they completed inpatient treatment. Results indicated that lowered VmPFC activity in response to stress exposure relative to the response when patients were relaxed was significantly associated with stress-induced alcohol craving and also predicted a shorter time to future relapse (see figure 1B) (Seo et al. 2013). In addition, lower VmPFC activity and insula response to stress was significantly correlated with more days of alcohol use during subsequent followup, emphasizing the contribution of altered stress neural circuitry to relapse susceptibility (Seo et al. 2013).

Although further work still is needed to fully understand the associations between stress-related neural response during abstinence, treatment, and early alcohol recovery, available data suggest that neuroadaptations in the peripheral and CNS involved in stress modulation play a significant role in recovery from chronic alcoholism. Altered emotional and stress responses and poor abilities to cope under stress observed in people with AUD may increase vulnerability to high-stress–related craving, relapse, and alcohol drinking, especially under challenging life circumstances.

PFC Regulatory Function in Alcohol Recovery

If repeated alcohol exposure disrupts the limbic-striatal system, the result could progressively debilitate prefrontal executive functions (Seo and Sinha 2014). Chronic alcohol-related PFC impairments, in turn, can compromise one's ability to recover from alcoholism by adversely influencing executive function, inhibitory control, and decisionmaking (Bechara 2005; Goldstein and Volkow 2011). Many neuroimaging studies consistently have indicated structural and functional deficits in prefrontal regulatory regions associated with chronic alcoholism (for review, see Buhler and Mann 2011).

Structural imaging studies, for example, find reduced gray matter volume in the medial PFC/OFC and its surrounding regions in AUD patients, and this is associated with poor treatment outcome. One study (Durazzo et al. 2011) examined cortical thickness in people with AUD who averaged 35 to 36 years of lifetime drinking and were seeking treatment. Patients who relapsed by a 12-month followup had decreased cortical thickness, especially in the OFC and right rostral/caudal middle frontal cortex, compared with AUD patients who continued to abstain after 12 months. (Durazzo et al. 2011). In an MRI study that examined AUD patients with an average of 18.6 years of alcohol use, soon after they became abstinent during treatment, patients had decreased brain volume in the gray matter of their medial PFC and posterior parietal-occipital area. At a 3-month followup after treatment, the researchers found that the degree of volume reductions significantly predicted a shorter time to initial relapse as well as heavy-drinking relapse, even after controlling for years of alcohol use and baseline alcohol intake (Rando et al. 2011). Furthermore, a study investigating both structural and functional patterns in detoxified AUD patients found that patients who relapsed within 3 months after completing treatment showed atrophy in regions of OFC and right medial PFC and ACC compared with patients who did not relapse. Relapsed patients also showed increased alcohol cue–induced activation in the left medial PFC regions in this study (Beck et al. 2012).

Functional neuroimaging studies also have reported connections between alcoholism, alcohol recovery, and altered activity in the medial PFC, OFC, and striatum. In AUD individuals, PET imaging studies found decreased glucose metabolism in the frontal cortex during alcohol withdrawal (for review, see Volkow and Fowler 2000) and reduced availability in striatal D2 receptors associated with lowered OFC/ACC function (Volkow et al. 2007). A study performing a 3-month followup on alcohol-dependent patients who had been abstinent for an average of 7 weeks prior to the start of the study, found that the five patients who relapsed showed pronounced activity in the medial PFC, ACC, and striatum when they viewed alcohol pictures. In these patients, there was a significant association between medial PFC activity and the amount of subsequent alcohol consumption (Grusser et al. 2004). Another study used SPECT to study brain blood flow in AUD inpatients at the end of an alcohol detoxification program that lasted at least 7 days. The nine patients who relapsed 2 months later displayed decreased blood flow in the medial frontal lobe and poor working-memory performance relative to 11 abstainers. The working-memory deficits were associated with low blood flow in the medial frontal lobe (Noel et al. 2002). These neuroimaging studies point to a potential significant role of structural and func-

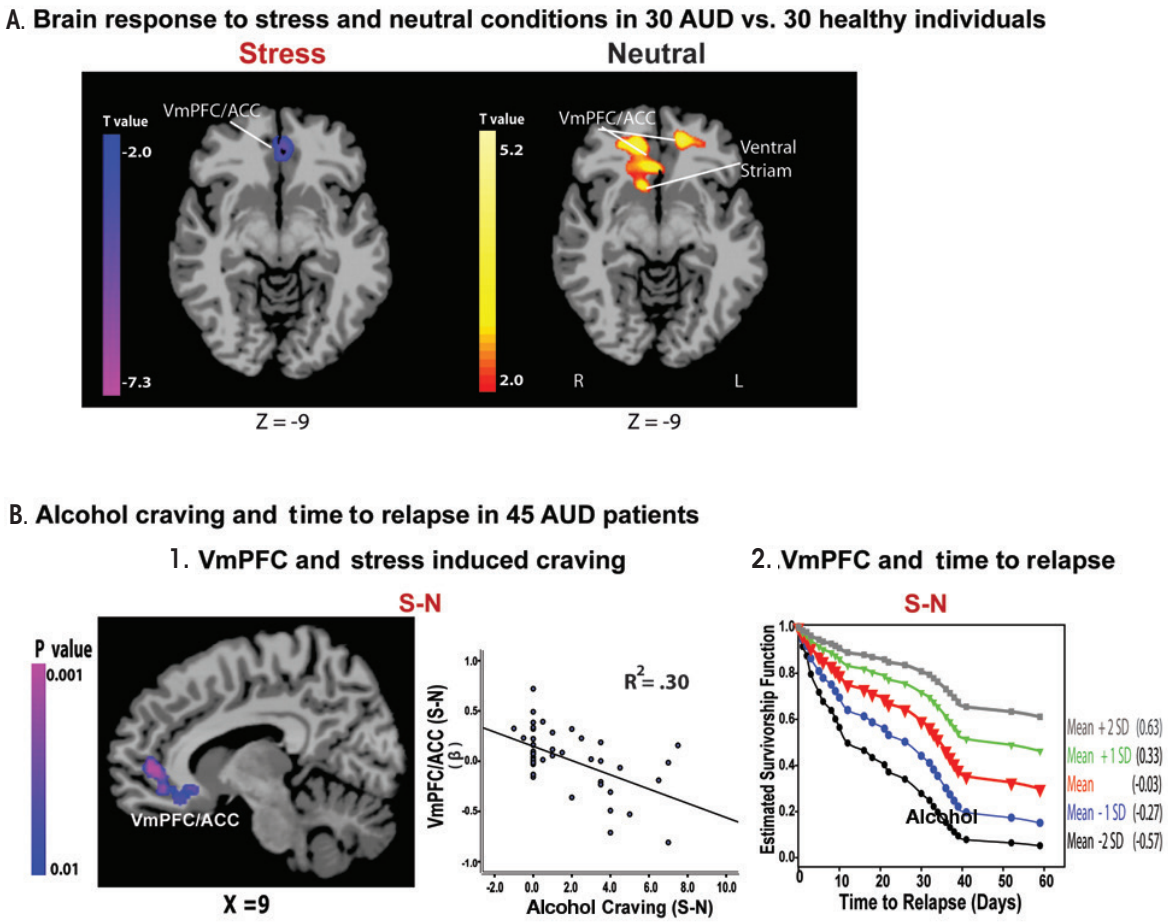

Figure 1 Hypoactive ventromedial prefrontal cortex (VmPFC) response to stress, alcohol craving, and relapse risk. **(A)** Hypoactive VmPFC response to stress but hyperactive response to neutral-relaxing condition in 30 patients with alcohol use disorder (AUD) compared with 30 healthy control subjects. AUD patients showed hypoactive VmPFC and anterior cingulate cortex (ACC) response to stress compared with demographically matched healthy control subjects ($P < 0.05$; whole-brain familywise error correction [FWE] corrected). **(B)** Neural correlates of alcohol craving and relapse in 45 AUD patients. **(B-1)** Whole-brain correlation analyses indicated that hypoactive VmPFC/ACC response to stress, compared with a neutral condition, was associated with increased alcohol craving during stress ($r = –0.55$; $R^2 = 0.30$; $P < 0.01$ whole-brain FWE corrected). No other regions were significantly associated with craving in this whole-brain voxel-based analysis. **(B-2)** Estimated survival functions for time to initial alcohol relapse are presented to illustrate the increasing risk of relapse with signal changes in the VmPFC hypoactivity during stress relative to the neutral condition: mean (in red) +1 (green) and +2 (gray) standard deviation (SD) above the mean, and –1 (blue) and –2 (black) SD below the mean. Cox proportional hazards regression analysis also indicates that hypoactive response during stress-neutral predicted a shorter time to initial alcohol use ($\chi^2 = 5.37$, $P < 0.05$; hazard ratio [HR] = 0.22, confidence interval [CI] = 0.06–0.79) as well as heavy-drinking relapse ($\chi^2 = 5.5$, $P < 0.05$; HR = 0.21, CI = 0.06–0.77). S-N = stress-neutral.

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tional neuroplasticity in the prefrontal regulatory regions involving the medial PFC, OFC, and ACC in increased relapse risk and poor alcohol recovery.

Neuronal Hyperexcitability and Alcohol Recovery

Recent evidence in humans suggests that excessive, chronic alcohol consumption may lead to hyperexcitability of neurons in the CNS, which, in turn, plays a role in alcohol addiction and recovery (Porjesz and Begleiter 2003; Seo et al. 2013; Sinha et al. 2011). Indeed, studies have found hyperactive CNS and electroencephalogram (EEG) responses in people with AUD, including increased excitatory neurotransmission associated with long-term alcohol use and hyperactive EEG responses in the frontal regulatory regions (Bauer 2001; Porjesz and Begleiter 2003). Studies also have found that alcohol-related neuronal adaptations on basal-state physiology, including upregulated ANS and HPA axis systems, underlie high alcohol craving, poor clinical outcome, and relapse vulnerability by disrupting physiological arousal (Breese et al. 2011; Seo et al. 2013; Sinha et al. 2011). In addition, a recent fMRI study found significant associations between hyperactive brain response during a relaxed state and susceptibility to alcohol craving and relapse in AUD patients who were engaged in treatment and 4 to 8 weeks abstinent. In this study, hyperactivity during relaxation in the VmPFC/ACC, but no other region, was associated with greater alcohol craving when subjects were presented with alcohol cues (figure 2A). In addition, the VmPFC/ACC hyperactivity predicted a shorter time to subsequent initial relapse and heavy-drinking relapse (figure 2B), as well as more alcohol use during a 90-day follow-up period (Seo et al. 2013). These findings highlight the important role of basal-state hyperactivity and integrity of VmPFC function in recovery from chronic alcoholism.

Conclusion and Future Directions

Alcoholism is a chronic illness, characterized by high relapse risk. Research now suggests that underlying this chronic relapse risk may be negative neuroplastic changes in the brain caused by the cycle of continued alcohol abuse and repeated brief alcohol abstinence and/or alcohol withdrawal. These neuroplastic changes occur in the PSL circuit, which regulates emotions and decisionmaking, which, in turn, influence alcohol recovery (Bechara 2005; Everitt and Robbins 2005; Goldstein and Volkow 2011). Within the PSL circuit, the PFC regulates limbic and striatal regions to modulate emotional and physiological responses to various

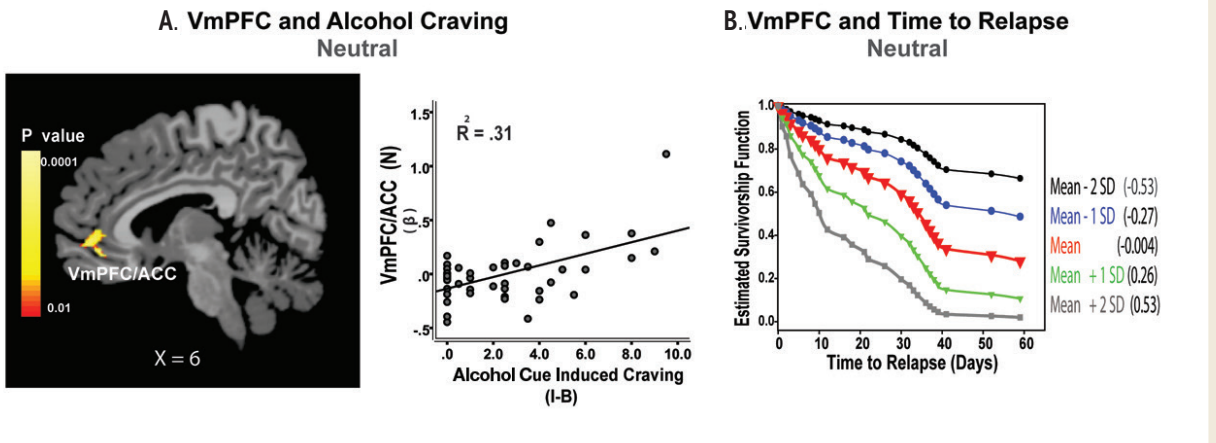

Figure 2 Hyperactive ventromedial prefrontal cortex (VmPFC) response to the neutral-relaxing condition, alcohol craving, and relapse risk. **(A)** In 45 patients with alcohol use disorder (AUD), hyperactive response in the VmPFC and anterior cingulate cortex (ACC) when they are exposed to neutrally relaxing situations during brief guided imagery was significantly associated with high alcohol craving during alcohol cue imagery ($R = 0.56$; $R^2 = 0.31$, $P < 0.01$ whole-brain FWE corrected). **(B)** Estimated survival functions for time to initial alcohol relapse, showing that the more VmPFC hyperactivity during the neutral condition, the shorter the time to subsequent initial relapse and heavy drinking relapse: mean (in red) +1 (green) and +2 (gray) standard deviation (SD) above the mean, and –1 (blue) and –2 (black) SD below the mean. Cox proportional hazards regression analysis indicates that hyperactive VmPFC response during the neutral condition predicted a shorter time to initial alcohol use ($\chi^2 = 6.39$, $P = 0.01$; hazard ratio [HR] = 8.45, confidence interval [CI] = 1.6–44.2) as well as heavy drinking relapse ($\chi^2 = 7.39$, $P < 0.01$, HR = 8.68, CI = 1.8–41.2). I-B = imagery minus baseline ratings.

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reward- and stress-related stimuli (Seo and Sinha 2014). In individuals with chronic alcoholism, persistent sensitization of subcortical limbic-striatal regions from prolonged alcohol use could compromise the PFC regulatory function, resulting in difficulties in emotional regulation, poor impulse control, and high alcohol craving. Substantially weakened PFC function could, in turn, further disinhibit limbic-striatal responses especially under challenging situations, including stress and exposure to alcohol-related cues. In addition, given the crucial role of the PFC in inhibitory control and decisionmaking (Bechara 2005; Goldstein and Volkow 2011), altered PFC function could result in an inability to inhibit compulsive alcohol seeking and poor decisionmaking when confronted with the choice to return to drinking and continued alcohol use despite negative consequences, thereby aggravating the relapse cycle.

The evidence supporting a role of the neuroadaptative changes in the PSL circuit in alcohol recovery points to important clinical implications:

- The neuroadaptive patterns in this circuit may serve as a set of neurobiological markers of alcohol relapse and recovery. Future research can validate these patterns and investigate their use to help predict relapse vulnerability and to identify people with the greatest challenge to alcohol recovery in the clinical setting.

- Researchers could develop and test novel treatment strategies that target these validated biomarkers and attempt to reverse these neuroadaptations and significantly improve the chances of recovery from alcoholism. Already evidence supports a mediating role of neuroplasticity in the PSL circuit in improving treatment outcome. A study (Muller et al. 2009) with deep brain stimulation showed the effectiveness of this method in recovering ventral striatal function in AUD individuals. In addition, a recent fMRI study (Brewer et al. 2011) showed that meditators with mindfulness training experience have stable VmPFC and posterior cingulate cortex activity compared with control subjects as well as stronger connectivity between cingulate cortex and dorsolateral PFC, suggesting that mindfulness training may hold potential for treating alcoholism. Consistent with these data, a recent clinical outcome study (Bowen et al. 2014) reported that participants assigned to a mindfulness-based relapse prevention program had fewer days of drug use and decreased heavy drinking, compared with cognitive–behavioral relapse prevention or 12-step–based program approaches at a12-month followup.

- Researchers could develop treatments that target withdrawal symptoms and stress-related pathology, such as stress-induced craving and alcohol seeking, implicated by the alcohol-related neuroadaptations in the PSL circuit. For instance, alpha1-adrenergic antagonists, such as Prazosin, show promise for improving stress-induced deficits and impaired PFC function from chronic stress (for a review, see Arnsten 2009). This drug also reduces alcohol withdrawal symptoms and stress-related alcohol seeking in animals (Kukolja et al. 2011; Walker et al. 2008) and improves stress and alcohol cue–induced craving and alcohol use outcomes in humans (Fox et al. 2012; Simpson et al. 2009). Alternative medicines, such as herbal remedies, are another area of interest. For example, Ge Gen (Kudzu root, Rx. Pueriariae), an herbal remedy frequently used in Eastern medicine, is effective in controlling alcohol intake and alcohol-related withdrawal symptoms (Benlhabib et al. 2004; Lukas et al. 2005). Studies that examine whether the treatments can restore PSL circuit function, especially the VmPFC, and improve alcoholism recovery rates, would be beneficial.

In conclusion, current neurobiological research in humans has identified neuroplasticity in the PSL circuit and its related dysfunctions as key factors increasing relapse risk and jeopardizing alcohol recovery. Further development of biomarkers for these alcohol-related neuroadaptive changes and new treatments that aim to restore the brain have the potential to influence the development of new treatment strategies to promote alcohol recovery and reduce the global burden associated with alcoholism.

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Neuroplasticity in Human Alcoholism

Studies of Extended Abstinence with Potential Treatment Implications

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Alcoholism is characterized by a lack of control over excessive alcohol consumption despite significant negative consequences. This impulsive and compulsive behavior may be related to functional abnormalities within networks of brain regions responsible for how we make decisions. The abnormalities may result in strengthened networks related to appetitive drive—or the need to fulfill desires—and simultaneously weakened networks that exercise control over behaviors. Studies using functional magnetic resonance imaging (fMRI) in abstinent alcoholics suggest that abstinence is associated with changes in the tone of such networks, decreasing resting tone in appetitive drive networks, and increasing resting tone in inhibitory control networks to support continued abstinence. Identifying electroencephalographic (EEG) measures of resting tone in these networks initially identified using fMRI, and establishing in longitudinal studies that these abstinence-related changes in network tone are progressive would motivate treatment initiatives to facilitate these changes in network tone, thereby supporting successful ongoing abstinence.

Key words: Alcoholism; alcohol use, abuse, and dependence; neuroplasticity; brain; brain networks; appetitive drive networks; appetitive drive; behavior control; inhibitory control networks; functional magnetic resonance imaging; abstinence; electroencephalographic; treatment

A person with alcoholism engages in risky or dangerous drinking despite experiencing serious negative physical and social consequences. Such persistence in pursuing damaging behaviors suggests that the short-term "appetitive" results of drinking (such as intoxication and losing one's inhibitions) have greater control over the alcoholic's behavior than do the negative consequences. From a neurobiological perspective, this pattern implies weak "top-down"—or knowledge-driven—executive control over impulsive and compulsive urges to consume alcohol and a strong "bottom-up"—or stimulus-driven—appetitive drive to consume alcohol, both impulsively and compulsively.

Research using functional magnetic resonance imaging (fMRI) has identified networks of disparate brain regions involved in executive control and others involved in appetitive drive. Studies in alcoholics have demonstrated differences in activity in these networks compared with nondrinkers, implying that the networks can contribute to the poor decisionmaking and risky behaviors seen among alcoholics. This article reviews fMRI evidence that, compared with non–substance-abusing control subjects (NSACs), brain executive control networks are weakened or "tuned down" and appetitive drive networks are strengthened or "tuned up" in active alcoholism. Further, alcoholism correlates with changes in synchrony, or how well the brain regions within each network operate in concert. We also present cross-sectional fMRI data showing that abstinence maintenance is associated with compensatory changes in synchrony in these networks, such that the executive control network has greater synchrony and the appetitive drive network has reduced synchrony both in comparison to NSACs. The article proposes that electroencephalographic (EEG) analogs of these alcohol-related network differences exist and should be characterized. EEG could reveal different properties of these brain networks, such as timing of event processing, and may be more amenable than

fMRI to active interventions such as neurofeedback. The article reviews a wide literature that supports the potential efficacy of an EEG neurofeedback intervention to mimic or augment the network changes seen in long-term abstinence. Finally, it presents a prototype showing that such neurofeedback is technically feasible.

Brain Network Activity in Alcoholics

To understand what brain changes underlie behavior seen in alcoholism, researchers have focused on two networks believed to influence whether a person acts to fulfill a desire or to govern or control the desire when faced with a choice. These two networks are the appetitive drive and executive control networks (see sidebar, "Brain Regions and Their Contributions to Behavior"). During its early stages, alcohol consumption is a goal-directed behavior, initiated and executed by regions within the executive control network (such as the dorsolateral prefrontal cortex and anterior cingulate cortex), with its rewarding effects processed by appetitive drive regions (such as the nucleus accumbens). After a person repeatedly consumes alcohol, consumption may become more automatic (with more involvement of appetitive drive regions such as the caudate and putamen) and less voluntary (with less involvement of executive control regions) (Everitt and Robbins 2005). Alcohol consumption shifts to a more habitual mode, particularly to avoid withdrawal symptoms. The behavioral fate of repetitive actions, such as compulsive alcohol consumption, seem to be instantiated in mesostriatocortical networks (Graybiel 1998; Volkow et al. 2013). An individual with alcohol dependence seeks alcohol compulsively—a behavior associated with increased activity of appetitive drive regions when presented with an alcohol cue—and experiences a lack of engagement of prefrontal regions, which under normal circumstances inhibit or stop a maladaptive behavior such as excessive alcohol consumption.

To determine how activity in these brain regions looks among alcoholics compared with control subjects, researchers use fMRI. fMRI measures brain activity by detecting the blood-oxygen-level–dependent (BOLD) contrast related to neural activity. Most fMRI experiments examine task-related patterns in the location and magnitude of the BOLD response, that is, the task activation of the brain. Many differences in activation in the executive control and appetitive drive networks have been observed in alcohol use, abuse, and dependence, suggesting that these networks and the multiple brain regions they encompass can contribute to the poor decisionmaking and risky behaviors seen in alcoholism (for a review, see Camchong et al. 2014).

For example, increased activity in the amygdala and insula, which are associated with inflexible, poor decision-making (Xiao et al. 2013), appears in binge drinkers. Lower activity in the dorsolateral prefrontal cortex (DLPFC) occurs among short-term abstinent alcoholics during inhibition tasks (Li et al. 2009) and in those with a family history of alcoholism during response inhibition (Norman et al. 2011) or when they are asked to make risky versus safe decisions (Cservenka and Nagel 2012). Further, lesser activation of prefrontal executive control regions compared with control subjects has been observed in alcoholics during spatial and verbal working-memory tasks (see the textbox on "Brain Regions and Their Contributions to Behavior") (Cservenka and Nagel 2012; Desmond et al. 2003; Pfefferbaum et al. 2001). Active drinkers show enhanced BOLD activation in the ventral striatum when presented with visual alcohol cues, which also supports the notion of a stronger appetitive and reward drive in people with current alcohol dependence (Ihssen et al. 2011; Myrick et al. 2004, 2008). Active drinkers with a diagnosis of alcohol dependence compared with active drinkers without alcohol dependence show higher activity in their DLPFCs when performing a delayed-reward decision task (Amlung et al. 2012). This increased activity may reflect increased demand that alcoholics (vs. NSAC) place on the executive control network when required to make decisions to delay behavior ruled by appetitive drive.

These studies demonstrate that excessive alcohol use and even the genetic vulnerability to alcoholism (observed prior to initiating alcohol use) is associated with activation patterns different from those of control subjects in brain regions that are part of the executive control and appetitive drive networks. More recently, scientists have taken fMRI studies a step further to examine differences in how well such brain regions work together. Such work suggests that faulty co-activation or synchrony within brain networks, or an imbalance between opposing brain networks, is important in alcoholism.

Synchrony in Brain Networks

Various methodologies for detecting brain activity demonstrate that more than one region becomes activated at a time, both during task performance and while at rest. Imaging studies now have begun to parse how the regions work together and whether disturbances within networks are associated with identifiable patterns of behavior. Early fMRI studies primarily focused on changes in the magnitude of the BOLD response, assessing activation and de-activation of brain regions during a task. More recently, studies have shifted to using fMRI to probe the similarity or synchrony of the BOLD response across spatially disparate regions, especially while the brain is at rest. The work builds upon the EEG literature that long ago established the existence of spontaneously oscillating brain networks. EEG measures brain electrical activity. Oscillations detected with EEG at characteristic frequencies, or bands, represent the

summed activity of thousands of neurons. Synchrony of oscillatory activity between brain regions is thought to support neural communication and plasticity (for review, see Fell and Axmacher 2011). For example, electrophysiological studies suggest that gamma band (higher than 30 Hz) synchronization is responsible for the integration of brain regions involved in specific aspects of stimulus processing. Synchrony of gamma oscillations enhances neural communication between regions, and lack of synchronization actually may prevent neural communication between cell assemblies. Scientists also have proposed that synchronization facilitates neural plasticity by enabling spike-field coherence that promotes the induction of long-term potentiation in neurons (see Glossary). Supporting this idea, studies show higher phase synchronization during encoding of information that a subject remembers than during encoding of information that the subject does not remember. Thus, scientists typically interpret high correlation or synchrony as representing a more integrated and responsive network and a low correlation or synchrony as representing a dysfunctional network or one with impaired communication. Network synchrony often is referred to in the literature as "functional connectivity."

Researchers largely agree that cortical oscillations evident in the EEG are related to the BOLD signal detected in fMRI, although the precise relationship is an area of active research (Thompson et al. 2014*a*,*b*; Whitman et al. 2013). This relationship suggests that changes in synchrony of the BOLD response may prove analogous to changes in synchronous EEG oscillations that reflect network integrity. Measuring network synchrony using fMRI can provide more precise information about the locations of brain regions acting together than EEG can capture.

Studies of the synchrony of the fMRI BOLD response during rest have gained in popularity, leading to the identification of several networks that are intrinsic to the brain's function (for review, see Lee et al. 2013). The most widely studied network is perhaps the default mode network (DMN), which is a group of brain regions that are active at rest but de-activated during cognitive tasks and which exhibits a highly synchronous low frequency (lower than 0.1 Hz) BOLD signal at rest. Many other networks that are highly synchronous at rest have been identified, including the somatosensory, visual, auditory, language, attention, and executive control networks. Networks identified during rest are robust, reliably detected in most people, and remain intact during task performance, although task synchrony may differ from synchrony observed during rest (Wilcox et al. 2011). The success of using synchrony measures in resting state fMRI has led to increased interest in measuring the synchrony of regions of activation in more traditional task-related fMRI studies. This work, in turn, has led to the identification of synchronous networks related to appetitive drive, cue salience, or behavior (Lee et al. 2013), which are key to studies of addiction.

Resting-State fMRI Synchrony Studies

Studies in Active Users and Very Early Abstinence

Because synchrony seems to represent the health of a network, it may be affected in certain networks by—or it may affect—alcoholism. Some recent work has examined resting-state fMRI synchrony in multiple brain networks in individuals with current alcohol use disorder (AUD) (Weiland et al. 2014). The fMRI time series measures of synchrony (i.e., average within-network correlations of BOLD signal magnitude across the network's nodes) were computed for 14 networks in each of 422 individuals with active AUD and in 97 control subjects. In this study, top-down executive control is reflected by the left and right executive control networks (LECN and RECN, respectively). The anterior salience network (composed of nodes including bilateral middle frontal gyrus, middle cingulate gyrus, and insula) reflects bottom-up appetitive drive. Network strength, a global measure of the fMRI time-series synchrony within each network, on average for all networks was lower for subjects with AUD than for control subjects. Tests of single networks showed lower synchrony in subjects with AUD versus control subjects for the LECN, consistent with the model that poor top-down executive control contributes to alcohol dependence. In addition, lower synchrony within the sensorimotor, basal ganglia, and primary visual networks in AUD versus control subjects may reflect alcohol's damaging effects on other networks that contribute to addiction. For the LECN alone, lower synchrony was associated with greater alcoholism severity and more years of drinking.

A study of fronto-striatal functional connectivity in cocaine use disorders supports the model that a strong bottom-up appetitive drive network is active in addiction (Wilcox et al. 2011). Fourteen subjects with chronic cocaine abuse or dependence (92% with comorbid alcohol abuse or dependence) in very early abstinence (but unlikely to be in significant acute withdrawal) had their resting-state fMRI recorded and compared with that of 16 healthy controls. Patients with chronic cocaine use exhibited increased synchrony between the ventral striatum and orbitofrontal cortex, key regions of the reward and appetitive drive network.

Studies in Long-Term Abstinence

The above section suggests that current dependence and abuse is associated with exaggerated bottom-up and compromised top-down neural network functioning. The question then becomes whether abstinence from alcohol changes that neural network

picture. Existing task studies suggest that compensatory mechanisms appear in long-term abstinence from nicotine and alcohol that may exert control over reward seeking and attenuate appetitive drive (Beck et al. 2009; Grüsser et al. 2004; Nestor et al. 2011; Wrase et al. 2007). To study brain network tone associated with long-term abstinence (LTAA), the authors examined resting-state fMRI synchrony in 23 LTAA subjects (8 women, ages 48.5 ± 7.1 years, abstinent 7.91 ± 7.80 years) and 23 NSAC subjects (8 women, ages 48.0 ± 6.7 years) (Camchong et al. 2013*b*). They used bilateral nucleus accumbens (NAcc) seeds (i.e., the fMRI time-series generated by the left and right NAcc) to probe the reward and appetitive drive network by identifying regions with synchronous fMRI responses, and a subgenual anterior cingulate cortex (sgACC) seed to probe the executive control network. All subjects also performed the intra-/extradimensional set shift task (IED; Cambridge Cognition 2006) outside of the scanner, and the study correlated their performance with the synchrony of the neural networks at rest. The IED assesses cognitive flexibility by examining an individual's ability to change a learned behavior with changing response contingencies.

Compared with NSAC subjects, LTAA subjects showed (1) decreased synchrony of limbic reward regions (e.g., caudate and thalamus) with both bilateral NAcc and sgACC seeds (figure 1) and (2) increased synchrony of bilateral NAcc seeds with left DLPFC (suggesting greater inhibitory control) and between the sgACC seed and right DLPFC (consistent with greater emotion regulation) (figure 2). The synchrony of bilateral NAcc seeds and left DLPFC was positively correlated with IED task performance outside of the scanner, suggesting that subjects with greater synchrony in the executive control network were better able to inhibit a learned response when a new rule was introduced. Additionally, duration of abstinence in LTAA was negatively correlated with the synchrony between sgACC and right DLPFC.

The lower synchrony of the limbic reward network in LTAA may reflect an ongoing compensatory effort to lower the induction of brain activity in regions known to be involved in reward processing. Increased synchrony between the NAcc and left DLPFC is consistent with literature showing that DLPFC input to the NAcc is involved in inhibition of behavior (Ballard et al. 2011; McClure et al. 2004), as is the correlation of this synchrony measure with IED performance.

LTAA subjects with a shorter duration of abstinence had higher synchrony between sgACC and right DLPFC. The authors suggest that individuals with shorter duration of abstinence are more vulnerable to relapse than individuals with longer abstinence and thus may need more vigilant emotional regulation (reflected here by increased synchrony between sgACC and right DLPFC) to manage emotional situations and successfully avoid relapse. On the other hand, individuals with longer abstinence, who are at lower risk for relapse, may have a lower need for regulating emotion; hence, lower synchrony between sgACC and DLPFC in LTAA subjects was associated with longer (multiyear) abstinence durations. In total, the results here support the existence of compensatory mechanisms in LTAA subjects that are evident during rest, in which enhanced synchrony within the executive control networks and attenuated synchrony within appetitive drive networks may facilitate the behavioral control required to maintain abstinence.

Studies of Comorbid Stimulant Dependence

To determine whether network synchrony abnormalities also underlie stimulant dependence, we examined LTAA subjects with comorbid stimulant dependence (LTAAS subjects; n = 35; 20 women, ages 47.9 ± 7.3 years; averaging 5.67 ± 4.80 years of abstinence), comparing them with 23 LTAA subjects without comorbid drug dependence (Camchong et al. 2013*a*) and 23 NSAC subjects. An earlier finding in this population shows that reduced activity in the insula (see sidebar, "Brain Regions and Their Contributions to Behavior") in stimulant addicts during decisionmaking (Paulus et al. 2005) or attention tasks (Clark et al. 2012) may predict subsequent relapse. Also, the insula has reciprocal connections with both the executive control (sgACC) and appetitive drive seeds (NAcc) (Craig 2009; Kelly et al. 2012), and accumulating evidence indicates insula involvement in behavioral aspects of addiction such as stress coping, decisionmaking, or cue responsiveness (Naqvi and Bechara 2010). The authors therefore examined synchrony of sgACC and NAcc seeds with insular activity in all three groups. The results showed commonalities in LTAA and LTAAS network synchrony. Compared with NSAC subjects, both groups showed enhanced executive control synchrony and enhanced synchrony between NAcc and midposterior insula. However, differences appeared as well. LTAAS subjects showed no attenuation of their appetitive drive network synchrony, with appetitive drive synchrony presenting higher in LTAAS subjects than LTAA subjects. LTAAS subjects also had enhanced synchrony between sg-ACC and the anterior or mid-insula compared with NSAC subjects. These findings implicate insula involvement in the top-down and bottom-up network adaptive synchrony phenomena in alcohol abstinence, especially in individuals with comorbid drug dependence. These results suggest common as well as specific targets for treatment to support abstinence in chronic alcoholics with, versus without, comorbid stimulant dependence. The results do not speak to possible similar effects in drug addicts without comorbid alcohol dependence, but suggest that studying

such individuals with the paradigms presented here may prove fruitful.

Studies in Short-Term Abstinence

Differences in synchrony observed among abstinent alcoholics compared with control subjects may reflect actual changes that the brain goes through to support abstinence or they may preexist in certain individuals and help those people to achieve and maintain abstinence. If the enhanced executive control network synchrony and suppressed appetitive drive network synchrony observed in LTAA subjects truly represent adaptive network changes during extended abstinence, then similar but smaller magnitude effects on network synchrony should appear in short-term abstinence. The authors investigated whether resting-state fMRI synchrony patterns found in LTAA subjects can be identified in short-term abstinent alcoholics (STAA subjects, abstinent 72.59 ± 18.36 days) (Camchong et al. 2013*c*). Using the same methodology as before (Camchong et al. 2013*b*), they examined network synchrony in 27 STAA subjects, and compared them with the 23 LTAA and 23 NSAC subjects from the previous study. They found synchrony effects ordered in magnitude from NSAC to STAA subjects and then to LTAA subjects within both the appetitive drive and executive control networks. Abstinence duration was associated with progressively lower synchrony of the appetitive drive network (NSAC subjects had higher appetitive synchrony than STAA subjects, who in turn had higher synchrony than LTAA subjects) and higher synchrony of the executive control network (NSAC subjects had lower executive synchrony than STAA subjects, while LTAA subjects demonstrated the highest level of executive control synchrony) (see figures 1 and 2). A significant positive correlation also appeared in STAA subjects between strength of synchrony between NAcc and left DLPFC and IED performance. Finally, the researchers saw a significant positive correlation in STAA subjects between strength of limbic reward network synchrony and current antisocial symptoms (i.e., antisocial behavior). These findings suggest that abstinent alcoholics experience adaptive differences in synchrony patterns compared with control subjects, and the magnitude of the difference increases with duration of abstinence.

Summary of Resting-State fMRI Synchrony Studies

These studies indicate that active alcoholics exhibit lower top-down executive control network synchrony and higher bottom-up reward and appetitive drive network synchrony, and that these phenomena are more than reversed with successful abstinence. The observed "overcompensation" in network synchrony—that is, the greater executive control network synchrony observed in STAA and LTAA subjects compared with control subjects—may be necessary in order to inhibit the habitual response to alcohol. This is consistent with the authors' 2013 paper showing that antisocial disposition does not change with long-term abstinence but that antisocial behavior is inhibited, with antisocial symptoms approaching zero in LTAA subjects (Fein and Fein 2013). Given this earlier observation of no change in antisocial disposition (or antisocial thinking) in LTAA subjects, it is not surprising that alcoholics need a very strong inhibitory control system to inhibit antisocial behavior (including drinking).

Task-Related fMRI Synchrony Studies

Several fMRI task studies have demonstrated altered executive control network activation and connectivity in alcoholism, implying that the resting-state fMRI synchrony differences observed are present during task processing. Research to determine the association between resting state fMRI network synchrony and network performance during tasks that involve appetitive drive and executive control would help demonstrate how the brain's readiness alters the brain's response to a task. For example, in nicotine addicts, a modified Stroop task (which tests the time it takes a subject to respond to a question about an image that contains nicotine versus neutral cues) has been used to assess appetitive drive and executive control networks (Nestor et al. 2011). The study provides evidence that higher executive control network activation when viewing nicotine cues occurs in former versus current smokers (i.e., higher executive control network activation appears with longer nicotine abstinence).

Jazmin Camchong developed an alcohol-cue analog of this task (see figure 3). In a pilot study, she tested five LTAA and two NSAC subjects who had demonstrated resting-state fMRI synchrony differences from each other. She found an alcohol-cue interference effect in LTAA subjects (i.e., longer reaction times to alcohol versus neutral cues) as well as higher synchrony of executive control regions in LTAA versus control subjects when viewing alcohol cues. These pilot results suggest that synchrony within the executive control network is higher in LTAA subjects both at rest and during task performance.

Task studies, which can isolate elements of complex behaviors, could help show not only whether synchrony influences behavior but what synchrony changes mean in relation to what scientists know about how alcoholism disrupts normal functioning. For example, one way of conceptualizing the core problem in alcoholism and other addictions is that reinforcements consequent to behavior—such as becoming sick or hungover after drinking—do not appropriately guide future behavior. Adaptive learning involves computation by the brain of reward prediction errors (PEs), which reflect the difference between expected

Brain Regions and Their Contributions to Behavior

Alcoholic behaviors represent a shift away from regulation of behavior by the brain's control and management functions (i.e., executive control) and toward influence by functions that process reward (i.e., appetitive drive). Parts of the brain's complex anatomy involved in each of these functions are spread far apart from one another. Nevertheless, they can act in concert to direct behaviors, and the balance between them turns out to have a profound impact in addiction and recovery. In the human brain, the appetitive drive and reward network—that is, the areas involved in forming and responding to appetites, drives, and desires—comprises mesocortico-limbic regions that mediate aspects of drug addiction such as responses to rewarding stimuli (e.g., the ventral tegmental area and nucleus accumbens), memory of rewarding stimuli (e.g., the amygdala and hippocampus), and regulation of emotion and executive function (e.g., the prefrontal and anterior cingulate cortices) (Everitt and Robbins 2005). The striatum (including the nucleus accumbens, ventral putamen, and ventral caudate) and orbitofrontal cortex are key regions mediating appetitive drive and behavior toward seeking reward (Elliott et al. 2010; Everitt and Robbins 2005; Taha and Fields 2006).

The subgenual anterior cingulate cortex (sgACC), a subdivision of the anterior cingulate cortex, plays a central role within the predominantly frontal cortical network underlying executive control (Botvinick et al. 2001). The ACC has widespread connections with the lateral prefrontal cortex and limbic structures (including the hippocampus, amygdala, and anterior thalamus) that are involved in emotional responsiveness and the regulation of behavior in the context of rewarding and punishing outcomes (Drevets et al. 1997; Kelly et al. 2009; Phan et al. 2005). A compromised top-down executive control network may underlie the poor regulation of behavior and emotion that has been considered primary in relapse (Berking et al. 2011; Cooper et al. 1995; Fox et al. 2008).

Here, images of the brain are labeled with some of the regions most important to the executive control and appetitive drive networks. The behaviors with which the regions are associated are also listed.

Appetitive Drive Network

Amygdala: *See* limbic system.

Caudate: Part of the striatum that influences goal-directed actions or behaviors.

Hippocampus: *See* limbic system.

Insula: Implicated in inflexible, poor decisionmaking. Also involved in stress coping and cue responsivity, which are behavioral aspects of addiction.

Nucleus Accumbens: Part of the striatum with roles in reward and reinforcement learning as well as fear, impulsivity, and addiction.

Orbitofrontal Cortex: Involved in motivational behavior as well as emotion and social behavior. It receives and responds to primary sensory information. It is involved in the detection and processing of consequences of behavior, including the attachment of emotional valence to the negative consequences of behavior.

Posterior Cingulate Cortex: Part of the default mode network (see Glossary) and possibly involved in human awareness. Also involved in pain and episodic memory retrieval and in intrinsic control networks.

Prefrontal Cortex: Involved in planning cognitive behavior, regulation of emotion, and executive function. Also part of the executive control network.

Putamen/Ventral Putamen: Part of the striatum involved in mediating appetitive drive. The putamen regulates movement and has influence on habits and on learning related to stimulus response.

Thalamus: *See* limbic system.

Ventral Tegmental Area: Involved in response to rewarding stimuli.

Executive Control Network

Basal Ganglia: Connected with the cerebral cortex, thalamus, and brain stem. Involved in the control of voluntary movement, procedural learning, habits, cognition, and emotion.

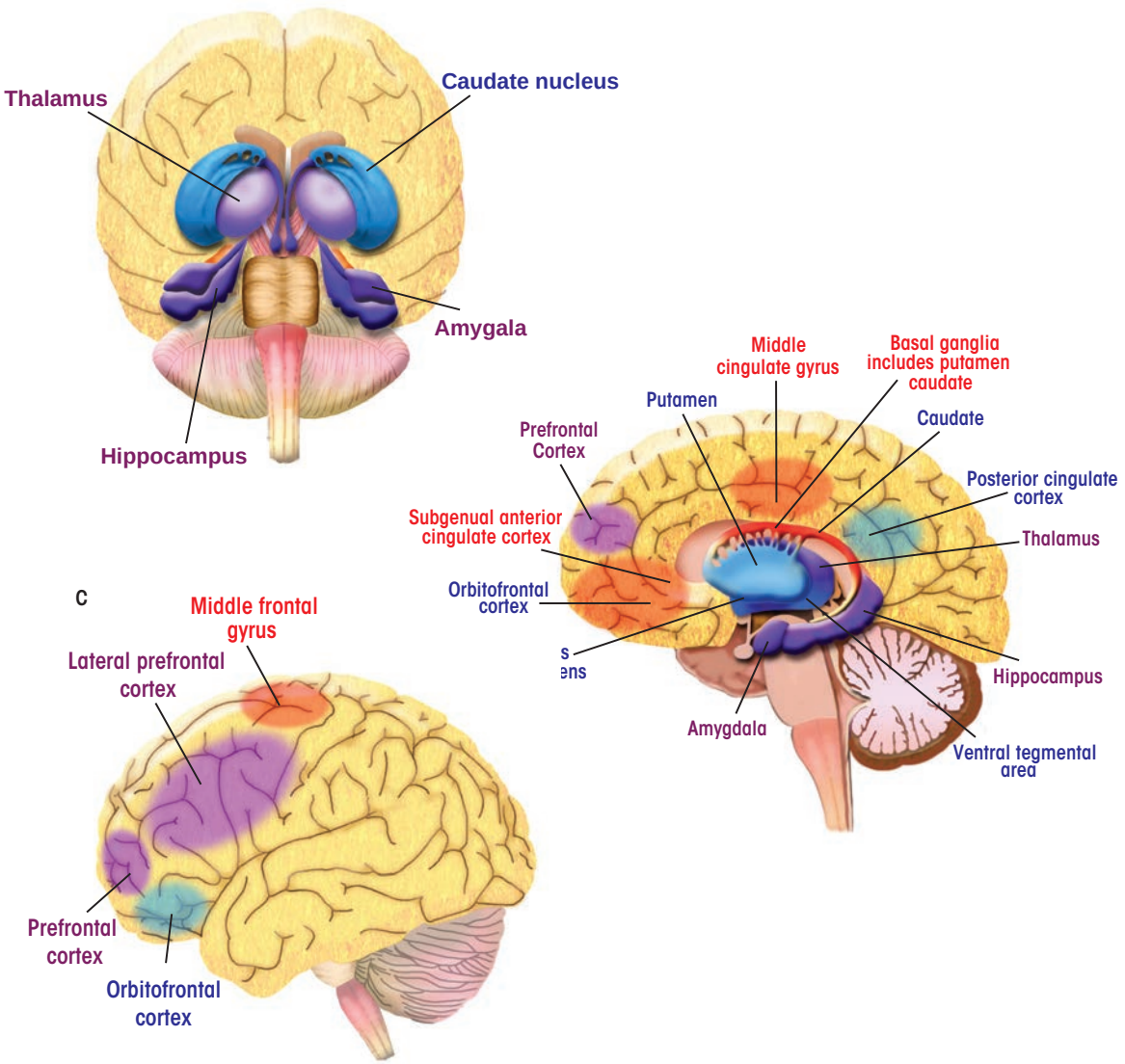
Bilateral Middle Frontal Gyrus/Middle Cingulate Gyrus: Parts of a salience network, a key mechanism by which the brain picks out details in its environment to focus on. Involved in learning and attention.

Lateral Prefrontal Cortex: Involved in goal-directed behavior. Includes the dorsolateral prefrontal cortex (a functional distinction), involved in executive functions such as working memory, cognitive flexibility, planning, inhibition, and abstract reasoning.

Limbic System: Encompasses the hippocampus, amygdala, and anterior thalamus. Implicated in both appetitive drive and executive control networks. An emotion, behavior, and motivation center.

continued

Brain Regions and Their Contributions to Behavior *(continued)*



Red–Executive Control Blue–Appetitive Drive Purple–Both Executive Control and Appetitive Drive

Locations of brain regions involved in executive control and appetitive drive. **(A)** Front Brain View: A frontal image of the brain showing internal structures involved in appetitive drive and in both appetitive drive and executive control networks. Though spread far apart in the brain's anatomy, the regions (shown here and in the other two brain illustrations) operate in concert to form these networks. **(B)** Side Brain View: A side view of the brain showing internal structures and locations of regions associated with either executive control or appetitive drive or, in many cases, with both networks. **(C)** External Brain View: An external view of the brain showing regions associated with the appetitive drive and executive control networks.

Brain Regions and Their Contributions to Behavior *(continued)*

continued

Subgenual Anterior Cingulate Cortex (sgACC): Connected with the lateral prefrontal cortex and with limbic system regions. Involved with emotion processing, learning, and memory.

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and actual outcomes. Normally, the PEs affect behavior by influencing higher-order executive functioning of the DLPFC, a region involved in goal-directed behavior. Park and colleagues (2010) tested models of the decision-making deficits in alcoholics and the networks underlying these deficits. They examined striatal PEs and functional connectivity between the striatum and DLPFC. A total of 20 male alcoholics in early abstinence (average 16.9 days abstinent) and 16 male healthy control subjects were studied using fMRI during a reward-guided decision-making task with changing response–outcome contingencies, which assesses how readily the subject learns. Alcoholics needed significantly more trials than did control subjects to meet learning criteria. In both groups, the PE from each stimulus presentation correlated significantly with the BOLD midbrain signal, and there were no differences between groups in the striatal PE signal. However, the influence of the striatal PE signal on the DLPFC was markedly attenuated in the alcoholics, suggesting that although the PE signal was being generated, it did not influence learning in the expected way. Moreover, striatal–DLPFC connectivity correlated significantly with learning during the task and was strongly negatively correlated with craving, especially in alcoholics.

In another study, 20 non–treatment-seeking problem drinkers underwent fMRI during a stop-signal task (SST) to assess response inhibition, a subject's ability to inhibit his own response to a stimulus (Courtney et al. 2013). Weaker functional connectivity between frontal regions and the striatum correlated with the severity of alcohol dependence, although SST behavioral performance was uncorrelated with severity, suggesting that the BOLD signal is more sensitive to alcohol's effects than task performance. The researchers concluded that as alcoholism progresses, the fronto-striatal pathway is weakened, leading to less inhibitory control as part of executive functioning.

Other studies of network functioning in alcoholics during active tasks have also revealed abnormalities in networks other than the executive control and appetitive drive networks. For some tasks, alcoholics seemed to recruit additional brain regions (vs. controls) to accomplish a task, perhaps to overcome strong appetitive signals, or physical or functional degradation of brain networks used by controls for task performance. Within the DMN, for example, abstinent alcoholics show less resting-state synchrony between the posterior cingulate and cerebellar regions compared with control subjects but show greater left posterior cingulate-cerebellar synchrony during a spatial working-memory task. The finding suggests that alcoholics need more integration of inputs from multiple brain regions to achieve comparable task performance to controls (Chanraud et al. 2011). In addition, higher connectivity among nodes of the DMN was associated with better task performance in both alcoholics and control subjects and also associated with longer abstinence in the alcoholics.

In later work (Chanraud et al. 2013), researchers observed that

compared with control subjects, recovering alcoholics recruited two additional fronto-cerebellar networks during a spatial working-memory task. In another study, lower fronto-cerebellar fMRI synchrony during a motor task also was observed in chronic alcoholics who were abstinent 5 to 7 days versus control subjects (Rogers et al. 2012). These results reinforce the idea that people generally require synchronous brain activity from disparate regions to respond appropriately to a stimulus and that alcoholics may need to marshal more brain regions to complete a task. The finding also provides evidence for improved network communication with extended sobriety.

A study of 18 abstinent alcoholics and 17 healthy control subjects acquired fMRI data during an attentional Stroop task (Schulte et al. 2012) and revealed abnormal synchrony in networks in the brains of abstinent subjects that may mediate between the top-down executive control and bottom-up appetitive drive networks. Using midbrain or posterior cingulate cortex (PCC) seeds (regions showing significant group-by-task activation contrasts in the fMRI analysis), the authors observed lower synchrony in alcoholics versus control subjects between the PCC and middle cingulate cortex, which they interpreted as reflecting difficulty in adapting functional network activity to executive task demands. They also observed greater synchrony between the midbrain and the middle cingulate cortex and striatal regions. They believe this suggests that alcoholics rely on greater integration of inputs from multiple brain regions as a compensatory mechanism to support task performance.

Task-related fMRI studies also may help identify characteristics of brain connectivity that can help predict whether or how readily an alcoholic will achieve abstinence. A cue-reactivity fMRI experiment with alcohol-associated and neutral stimuli was used to study 46 detoxified alcohol-dependent patients (19.74 ± 22.66 days abstinent) and 46 control subjects (Beck et al. 2012). Three months following scanning, 30 patients had relapsed and 16 had maintained alcohol abstinence. The study compared fMRI results of the subsequent relapsers with those of the abstainers. When presented with alcohol-associated versus neutral stimuli, abstainers had demonstrated stronger functional connectivity than those who had relapsed between midbrain (including the ventral tegmental area and subthalamic nuclei) and left amygdala and between midbrain and

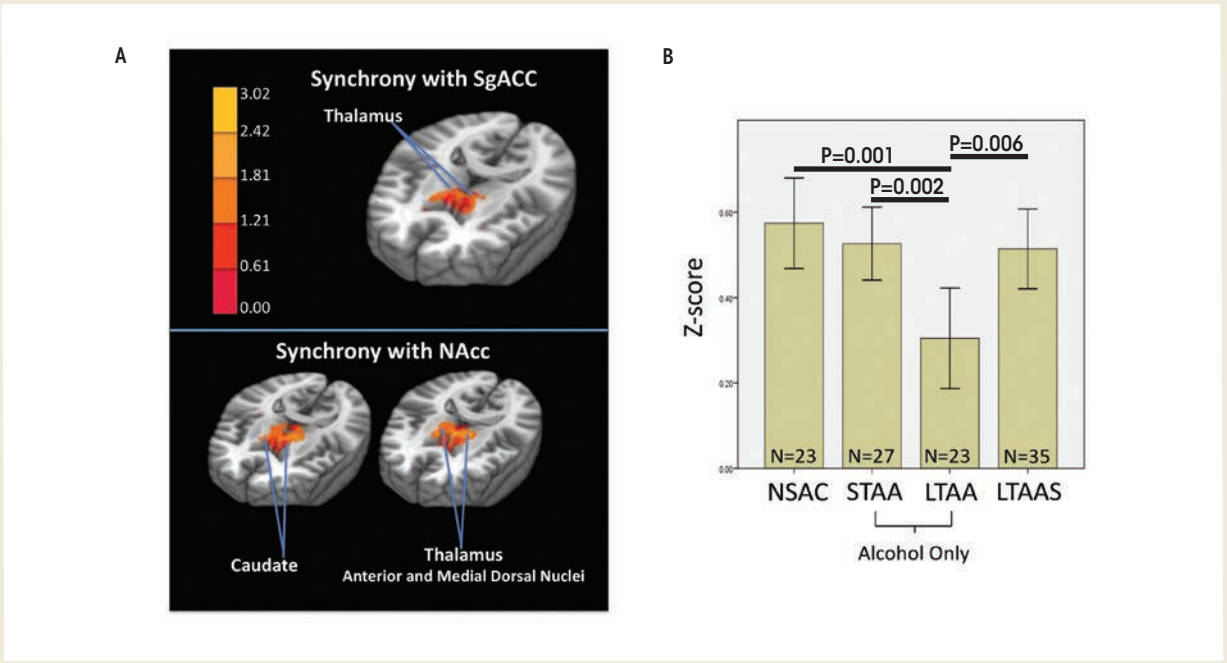

Figure 1 fMRI resting-state synchrony within the appetitive drive network is shown. **(A)** The voxels with activity synchronous to the subgenual anterior cingulate cortex (sgACC) and nucleus accumbens (NAcc) seeds are overlaid in red/yellow. These regions of the thalamus and caudate are crucial in bottom-up appetitive drive. (**B**) The average Z-scores indexing synchrony between the SgACC and NAcc seeds and the colored regions shown in the left panel are shown for non–substance-abusing control subjects (NSAC), short-term abstinent alcoholics (STAA), long-term abstinent alcoholics (LTAA), and stimulus-dependent long-term abstinent alcoholics (LTAAS). The LTAA show significantly less synchrony than NSAC, STAA, and LTAAS, with STAA and LTAAS synchrony midway between NSAC and LTAA.

left orbitofrontal cortex. These are brain regions associated with the processing of salient or aversive stimuli. The increased synchrony in abstainers between the midbrain and amygdala may mediate an enhanced aversive reaction to alcohol stimuli, which may then act as a warning signal (through stronger midbrain-frontal cortex synchrony) to help maintain abstinence.

In summary, fMRI functional connectivity or synchrony studies provide ample evidence that altered network synchrony exists in alcoholism and that plastic changes in network synchrony occur with abstinence. However, from cross-sectional studies alone, one cannot distinguish between brain synchrony actually changing in long-term abstinence (Camchong et al. 2013*b*,*c*), versus selective survivorship (i.e., individuals with such synchrony differences are more likely to achieve abstinence, and individuals with the largest differences from NSAC are more likely to achieve protracted abstinence), or a combination of the two. Only longitudinal studies can determine whether the observed cross-sectional findings indeed reflect adaptive changes in network synchrony with extended abstinence.

Applying Synchrony Findings to Treatment

Scientists understand little of how successful treatments such as behavioral therapies or 12-step programs work. They also understand little of the neurological mechanisms underlying reduction or cessation of drinking. Data reviewed here point to one such possible mechanism. They reveal network synchrony changes detected using fMRI that are graded with abstinence duration, suggesting that achieving and maintaining abstinence is associated with adaptive brain network synchrony changes that support reductions in bottom-up appetitive drive and increases in top-down executive inhibitory control. If longitudinal studies can confirm that the degree of the changes in the appetitive drive and executive control networks is associated with and predictive of successful abstinence, then such changes may underlie the success of behavior therapies. In addition, interventions that directly augment the network changes

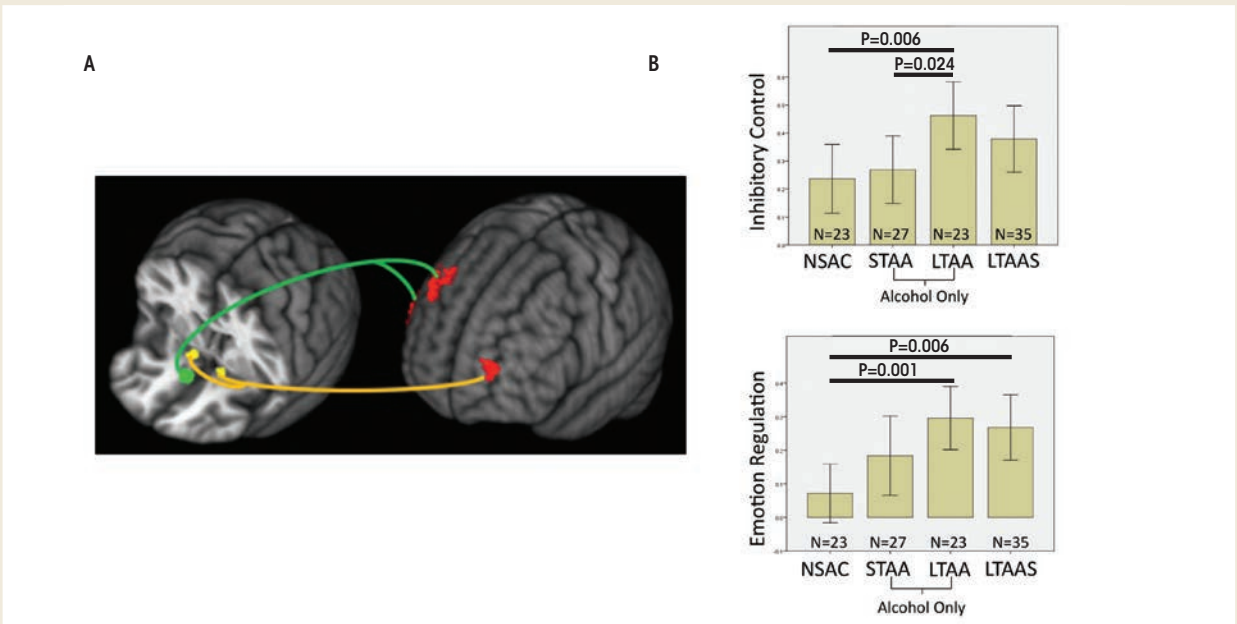

Figure 2 fMRI resting-state synchrony within the executive control network is shown. **(A)** The voxels with activity synchronous with the subgenual anterior cingulate cortex (sgACC, shown in green on the left brain image) are located in the right dorsolateral prefrontal cortex (DLPFC) and are overlaid in red on the right brain image. The voxels with activity synchronous with the bilateral nucleus accumbens (NAcc, shown in yellow) are located in the left DLPFC and are overlaid in red on the right brain image. The right DLPFC is associated with emotion regulation, and the left DLPFC is associated with inhibitory control. **(B)** The average Z-scores indexing synchrony between the NAcc and left DLPFC (top) and between the sgACC and right DLPFC (bottom) are shown for non–substance-abusing control subjects (NSAC), short-term abstinent alcoholics (STAA), long-term abstinent alcoholics (LTAA), and stimulus-dependent long-term abstinent alcoholics (LTAAS). The LTAA show significantly greater synchrony than NSAC and STAA, with STAA and LTAAS synchrony values slightly greater than NSAC, between inhibitory control brain regions. Both LTAA and LTAAS show significantly greater synchrony than NSAC, with STAA values midway between NSAC and LTAA, between emotion regulation brain regions.

may provide another tool in the treatment toolbox.

The idea of modifying brain network synchrony to promote abstinence is bolstered by the literature on using transcranial direct-current stimulation (tDCS) or repetitive transcranial magnetic stimulation (rTMS) to treat alcohol craving. These noninvasive treatments are thought to reduce craving by modulating the activity and connectivity of brain networks. Boggio and colleagues (2008) showed that tDCS of the DLPFC decreased alcohol craving compared with sham treatment. In later work, Mishra and colleagues (2010) studied 45 alcohol-dependent patients administered rTMS of the DLPFC and found significant decreases in a craving measure within the group that received rTMS compared with the sham group. One interpretation is that these treatments resulted in increased DLPFC activity and better executive control over craving.

A case study by De Ridder and colleagues (2011) provides further evidence that brain functions in alcoholism can be trained or influenced using relatively noninvasive techniques. The researchers used rTMS targeting the anterior cingulate cortex in an attempt to reduce craving and promote abstinence in a woman with a long history of alcohol dependence and treatment. Before treatment, the patient showed increased EEG synchrony between the ACC and PCC, and fMRI showed activation of regions of the appetitive drive network (NAcc, ACC, and PCC) in response to cue-induced worsening of craving. Following successful rTMS, fMRI-detected activation of NAcc, ACC, and PCC disappeared, and the patient's synchrony pattern normalized. When rTMS treatment became ineffective and relapse occurred, activity and synchrony within the appetitive drive network returned. Although their effect was not permanent, the rTMS treatments seem to have altered network synchrony and reduced craving.

Direct currents and magnetic waves applied transcranially thus seem to influence brain synchrony and may help reduce symptoms such as craving in alcoholism. At the same time, people can achieve abstinence without them. A technique such as neurofeedback might help people with addictions directly strengthen the tone of their inhibitory networks or weaken the tone of their appetitive drive networks. Neurofeedback is a method built upon the idea that the mind and body are one, and that by training the mind or brain to achieve particular states indexed by some measured neurobiological signal (such as the BOLD response or EEG), the body will react in a more optimal way in order to improve emotional, cognitive, physical, and behavioral experiences. Neurofeedback that "feeds back" an auditory or visual signal that corresponds to the strength of brain network synchrony may promote network synchrony adaptations that support abstinence. For example, a neurofeedback protocol may instruct a patient to try to raise the pitch of a tone. A low-pitched tone is played when network synchrony is low, and the pitch increases with network synchrony. As the patient works to raise the tone, synchrony in the target network improves, training the network.

Relating fMRI to EEG

Some technical challenges stand in the way of neurofeedback. First, it is neither practical nor economically feasible to use neurofeedback to modify fMRI-detected network synchrony directly. Furthermore, fMRI's BOLD response cannot provide the time resolution necessary to allow real-time feedback to a patient about synchrony changes occurring in his or her brain. In contrast, EEG provides precise time resolution and generally is a more economical and efficient tool for use in treatment than fMRI.

Since research on brain networks involved in alcoholism has used fMRI to date, scientists need to find EEG results that are analogous to the relevant fMRI-detected network phenomena to make EEG useful in neurofeedback. Fortunately, converging evidence suggests that the fMRI BOLD response reflects the summed neural activity of several oscillatory EEG networks (for review, see Whitman et al. 2013). These EEG networks may oscillate out of phase (i.e., the peak of oscillation does not coincide across nodes of the network) at multiple frequencies (e.g., theta, alpha, or gamma), and the activity of separate networks may vary as a function of cognitive states lasting only a few hundred milliseconds. fMRI networks detected in response to task processing are likely to comprise multiple oscillatory EEG networks reflecting both evoked (i.e., time-locked to the task) and induced (i.e., not time-locked) EEG responses and including responses that derive from phase alignment within EEG networks, wherein the summed activity creates a large, detectable signal (Burgess 2012). Because of the more complex nature of EEG measures of brain activity that change at the same pace as cognitive processes, EEG networks representing executive control and appetitive drive could potentially reveal more about the mechanisms underlying the processing and inhibition of alcohol cues that contribute to the maintenance of abstinence. Such EEG networks also could serve as neurofeedback targets.

Neurofeedback of EEG Network Synchrony

EEG network connectivity analysis is in its early stages, but pursuit of the identification of EEG networks that change with abstinence is crucial given the possibility of a neurofeedback intervention to facilitate abstinence. Preliminary data show that resting EEG coherency carries information that differs between LTAA and NSAC subjects, and that correlates with resting-state fMRI executive control network synchrony. Further study could identify reliable EEG executive control and appetitive drive network

synchrony measures as neurofeedback targets.

Roberto Pascual-Marqui's keynote address at the International Society for Neurofeedback and Research in 2011 presented a model for examining brain network synchrony from scalp-recorded EEGs. Using low-resolution electromagnetic tomography (LORETA) (Pascual-Marqui 2002, 2007) to estimate cortical EEG sources and independent components analysis (ICA) to identify synchronous source activity, he demonstrated EEG networks involving similar cortical regions to those identified by resting state fMRI from the literature. More recent work used EEG to study the effect of acute alcohol intake on the brain's resting state network in social drinkers. It examined the coherence between the activity of certain cortical areas within different frequency bands (Lithari et al. 2012) to construct brain networks. The work demonstrates that network synchrony changes occur over a short period of time (within 25 minutes of alcohol consumption) and are reflected in the scalp-recorded EEG, which can then be attributed to brain locations for network analysis. These results support the idea that EEG brain network synchrony could provide a neurofeedback target.

History

EEG neurofeedback in the treatment of substance use disorders dates to 1975 (for review, see Sokhadze et al. 2008) and was based on an alpha-theta training protocol, aimed at increasing the proportion of alpha (8 to 13 Hz) and theta (4 to7 Hz) band activity in the ongoing EEG to promote a state of profound relaxation similar to a meditative state. Although early studies were uncontrolled and abstinence rates were not reported, results suggested that biofeedback-induced alpha/theta states promoted insight and attitude changes in alcoholics, and that these changes enhanced recovery (Twemlow and Bowen 1976, 1977; Twemlow et al. 1977). Peniston and Kulkosky (1989) conducted the first randomized controlled studies of alpha-theta EEG neurofeedback. Of 10 alcoholic patients (who had formerly failed hospital treatment for alcoholism) who underwent neurofeedback training, 8 remained generally abstinent for at least 3 years, and they showed persistent changes in alcoholic personality variables. A case study (Fahrion et al. 1992) further described neurofeedback treatment in an 18-month-abstinent alcoholic who was experiencing craving and a fear of relapse. It concluded that neurofeedback was a useful intervention for reducing craving even in abstinent alcoholics. Later work also reported sustained abstinence in a group of alcoholic depressed patients who were treated with alpha-theta neurofeedback (Saxby and Peniston 1995). Critics deem alpha-theta neurofeedback no more effective than suggestion or meditation techniques. However, the fact that feedback of a single electrode measuring alpha and theta—which affords a limited view of the complex interaction of brain networks involved in alcohol abuse and dependence—works as well as it does, encourages the notion that feedback of EEG signals reflecting the functioning of the executive control and appetitive drive networks would yield even more impressive results.

To examine this idea that neurofeedback learning would be improved if activity from specific brain regions related to the desired outcome behavior was monitored, Congedo and colleagues (2004) pioneered neurofeedback using LORETA with a protocol

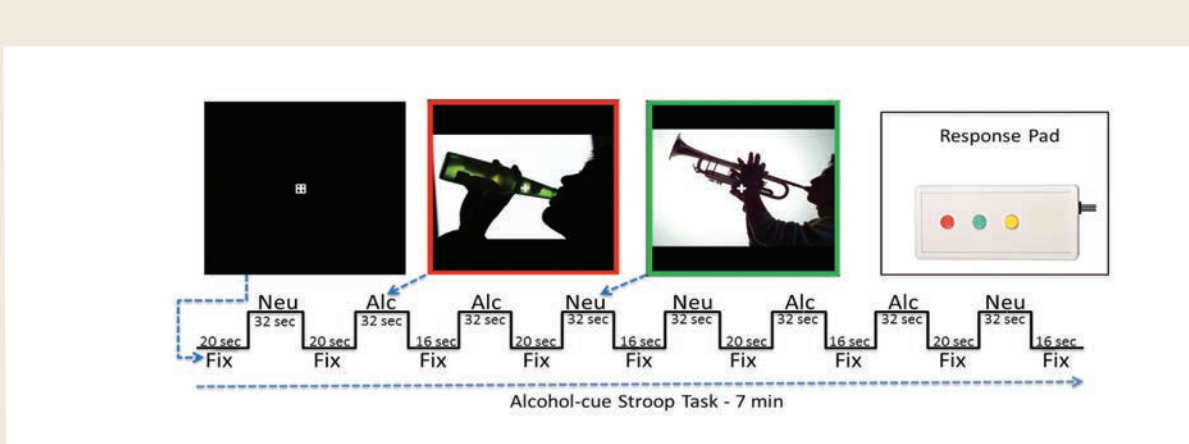

Figure 3 Alcohol-cue Stroop task. During the fixation (Fix) blocks, subjects keep their eyes fixated on the cross. During the neutral (Neu) and alcohol (Alc) blocks, subjects are instructed to keep looking at the fixation cross in the middle, while they notice the color of the picture's border, and respond by pressing the corresponding colored button on the response pad.

designed to improve sustained attention. Alpha and beta band current densities were estimated for an anterior cingulate region of interest using LORETA based on 19 scalp electrodes, and the power ratio between bands was used to drive feedback signals. They demonstrated that the current density power ratio increased over multiple neurofeedback sessions and that subjects could willfully increase that ratio. Scientists subsequently used LORETA neurofeedback to train eight healthy individuals to increase their low-beta power activity (moving the EEG frequencies in a direction opposite to alpha/theta feedback) for an anterior cingulate ROI in an effort to improve alertness and attention (Cannon et al. 2007). The subjects increased their beta power within the target ROI after neurofeedback, and these changes were associated with behavior change. Furthermore, beta power increases also were observed within ROIs that encompassed the left and right prefrontal cortex and the right post central gyrus, demonstrating parallel modifications in regions of the executive control network, although training targeted only a single anatomical node. More recent work has explored the feasibility of neurofeedback using a LORETA-derived anatomical source in clinical populations (Cannon et al. 2008) and has explored the utility of measuring EEG network synchrony using LORETA-derived sources (Cannon et al. 2012; Coben et al. 2014).

EEG Neurofeedback

The authors propose that EEG neurofeedback promoting increased inhibitory control network synchrony and reduced appetitive drive network synchrony would result in a "resting-state brain" that can more appropriately deal with the challenges of maintaining abstinence. The design of such an EEG neurofeedback protocol requires identification of EEG networks that change with abstinence and correspond to the

Cognitive Testing Tools

Alcoholism affects an array of cognitive functions that involve different brain regions. Asking a patient or subject to perform tasks that isolate specific cognitive processes from each other provides an essential tool for imaging studies, because the tasks induce measurable activity in the specific brain regions required to perform them. Researchers can compare activity patterns seen among alcoholics with those seen among abstainers and healthy control subjects. Tests referred to in this article are described here:

Delayed Reward Task: This tests a subject's ability to resist the temptation of an immediate reward in favor of waiting for a later reward. The task involves impulse control and self-control.

Intra/Extradimensional Set Shift Task: This tests the subject's ability to learn a rule through trial and error and then reverse it in favor of a new rule. The task requires attention and flexible thinking.

Motor Task: The task tests a subject's ability to learn and voluntarily produce intentional movements to proficiently perform a goal-oriented task. Motor tasks require considerable cognitive input.

fMRI Reward-Guided Decision-Making Task: This assesses a subject's learning rate by letting the subject look at different stimuli and choose one that is associated with a positive outcome (e.g., a smiley face). Each time the subject chooses an item and receives negative feedback (e.g., a frowning face), a prediction error is generated. The learning rate counts the number of trials the subject goes through to figure out which stimulus leads to a positive outcome.

Spatial and Verbal Working-Memory Tasks: Working memory actively holds multiple pieces of information in the mind where they can be manipulated. It includes subsystems that store and manipulate both visual images and verbal information. Tasks that test working memory require a subject to manipulate information as part of a goal-directed action while also being presented with distractions. The cognitive processes required to accomplish the task include executive control and attention, among others.

Stop-Signal Task: Here, a subject is asked to respond as quickly as possible to a particular feature of a stimulus (e.g., color, shape, or location). In some instances, however, the stimulus is followed by another signal—such as an auditory tone—that tells the subject to withhold her planned response. This tests the subject's ability to inhibit responses.

Stroop Task: This assesses whether a subject experiences interference in reaction time for completing a task. The classic Stroop test example involves looking at the names of colors spelled out in ink that is not the same color as the word (e.g., the word "red" spelled in blue ink). The subject is asked to name the color of the ink, and reaction time can indicate whether a person has problems with selective attention, cognitive flexibility, or processing speed.

appetitive drive and executive control networks previously identified using fMRI. Given the success of LORETA for estimating EEG network synchrony (Cannon et al. 2012; Coben et al. 2014; De Ridder et al. 2011) and the active research in the estimation of EEG sources and source synchrony (Chiang et al. 2009; Cook and Koles 2006; Gramfort et al. 2013; Sekihara et al. 2001), these networks likely can be identified and used as neurofeedback treatment targets for abstinence maintenance.

Technical challenges are inherent in a real-time EEG brain network synchrony neurofeedback system. However, the authors' prototype for an EEG neurofeedback system uses a quad-core Intel i5 computer to acquire EEG and estimate network synchrony based on comparing the EEG of each possible pair of electrodes, and a second computer to display a movie as the feedback signal. Although the best estimates of EEG network synchrony likely will be derived from intracranial source estimates, the prototype has computational demands greater than those required to estimate intracranial source connectivity and thus is more than adequate to establish the feasibility of a future EEG network synchrony neurofeedback system. The prototype records 64 channels of scalp EEG, estimates pairwise cross-coherencies, and computes the contribution of the independent components (IC) that index executive control or appetitive drive network synchrony. First, the subject's baseline network synchrony is estimated for use during training. During neurofeedback training, the system continuously records 64 channels of scalp EEG and analyzes the EEG to estimate network synchrony in real time. The real-time synchrony is compared with the subject's baseline synchrony and the target distributions of synchrony for NSAC, STAA, and LTAA subjects. A degraded video stimulus feeds back to the subject if there is a large difference between the real-time estimate of synchrony and the target synchrony, whereas a clear video signal appears when the real-time synchrony estimate approaches the target synchrony. The prototype is fast enough to update the neurofeedback to the patient 10 times per second despite a computationally intensive method of reflecting EEG network synchrony. It is likely that a much simpler algorithm will sufficiently index EEG network synchrony once research clarifies which signals best represent key aspects of brain network synchrony in recovering alcoholics. For example, neurofeedback systems could eventually use cross-correlation of selected electrode pairs within one or two frequency bands, or correlation of estimated source activity or power between a small number of anatomical sources. The central research task that would enable development of an EEG neurofeedback system to treat alcoholism remains identifying the EEG measures of network function that change with abstinence and that correspond to the appetitive drive and inhibitory control fMRI networks.

Conclusions

Alcoholism is characterized by a lack of control over excessive alcohol consumption despite significant negative consequences, a pattern of behavior that implies weak top-down executive control over impulsive and compulsive urges to consume alcohol, and a strong bottom-up appetitive drive that produces those urges. fMRI studies have identified multiple brain regions that contribute to the poor decisionmaking and risky behaviors seen in alcoholism. This chapter reviews fMRI network synchrony, or functional connectivity, studies suggesting that faulty coactivation or synchrony of multiple brain regions comprising networks, or an imbalance between opposing brain networks, is important in alcoholism. fMRI network studies in active alcoholics suggest that impulsive and compulsive behaviors are related to the ineffectiveness of brain networks, characterized by decreased synchrony in top-down executive control network and increased synchrony in the bottom-up appetitive drive network. Repeated high-volume alcohol exposure may compromise network integrity, as suggested by the relationship between synchrony and the severity and duration of alcohol use. Continued abstinence following alcoholism displays a different synchrony pattern. A series of studies in short- and long-term abstinent alcoholics observed decreased synchrony in appetitive drive networks and increased synchrony in inhibitory control networks, suggesting that the alcohol-induced imbalances in brain networks are reversed, helping individuals achieve and maintain abstinence by inhibiting behavior and reducing appetitive drive. Longitudinal studies of abstinent alcoholics at rest and during task performance would definitively establish whether plastic changes in the synchronous activity in brain networks reflects a crucial brain mechanism underlying the behavior changes in alcoholics that result in extended abstinence. Furthermore, the identification of EEG measures analogous to fMRI-executive control and appetitive drive network synchrony could potentially reveal the sequence and timing of mechanisms underlying the processing and inhibition of the brain's response to alcohol cues that contribute to the maintenance of abstinence. Confirming the progressive network synchrony changes with longitudinal studies of abstinent alcoholics—together with identifying EEG networks—would support the treatment potential of interventions to augment these network changes. Neurofeedback of EEG alpha and theta rhythms has been a successful component of alcoholism treatment in some subjects, and feedback of a signal that indexes synchrony in specific brain networks holds great promise as an alcoholism treatment. A prototype for neurofeedback to alter measures of EEG network synchrony demonstrates the technical feasibility of this treatment approach. If longitudinal studies

Glossary

Default mode network (DMN): A network of defined brain regions that is active when a person is not focused on the outside world but is awake. It is characterized by neural oscillations (see electroencephalography [EEG], below) and is deactivated when a person focuses on a task or action.

Electroencephalography (EEG): Records electrical activity along the scalp. EEG measures voltage fluctuations resulting from activity in the neurons of the brain. It can detect neural oscillations, which reflect the naturally occurring rhythmic, repetitive neural activity that occurs in the central nervous system. When many neurons act together, the synchronized activity results in the oscillations. Different synchronized activity between neurons gives off oscillations at different, characteristic frequencies. These frequencies have been aggregated into bands that have been named with Greek letters (e.g., alpha, theta, gamma, etc.).

Functional Magnetic Resonance Imaging (fMRI): A technique for assessing brain activity by measuring changes in blood flow that occur in response to neural activity (also called the BOLD response). MRI uses an electromagnet to align atomic nuclei, which then give off a measurable signal. MRI measures the magnetic signal from hydrogen nuclei in water. When neurons increase their activity, their demand for oxygen increases and blood flow increases to the area, allowing the system to determine what brain regions are active versus others.

fMRI Seeds: Signals from very precise locations in the brain region or structure of interest. Seeds can be a single fMRI volume element or a "region of interest" or ROI. Seeds are used to calculate correlations with the activity of all other locations, which appear as connections "growing" from the "seed," resulting in detailed data on connectivity in brain areas.

fMRI Task-Related Studies: Record and measure activation of brain regions while a subject is asked to complete a task, such as looking at a picture, that elicits a specific cognitive response in the brain. Studies can be designed so that the process of interest can be measured separately from other processes (see textbox).

Low-Resolution Electromagnetic Tomography (LORETA): A method for determining the location of electrical activity in the brain using multiple channel electroencephalography recordings.

Neurofeedback: Biofeedback that uses real-time displays that are a function of brain activity—including electroencephalography—to teach self-regulation of brain function.

Phase Synchronization: Occurs when a certain characteristic of an oscillation—the phase—is aligned in separate brain regions. When oscillations are in phase or synchronized, they reinforce each other.

Repetitive Transcranial Magnetic Stimulation (rTMS): Uses precisely targeted magnetic pulses to stimulate areas of the brain.

Spike-Field Coherence: Measures neuronal synchronization across brain areas by comparing spikes—electrical signals that occur when a neuron "fires" or emits an action potential—with the surrounding field potential, which is the compound activity of a large pool of neurons that may oscillate at different frequencies. Spikes may be synchronized or "coherent" at some frequencies that contribute to the local field potential, but may have no phase relation to other frequencies.

Synchrony: Oscillatory activity of physically distant brain regions occurring at the same time (or coinciding). Synchronization has been linked to cognitive functions.

Transcranial Direct Stimulation (tDCS): Uses constant, low current to stimulate a brain region, delivered to the brain region through scalp electrodes.

confirm that the adaptive changes in brain functional organization summarized in this article support ongoing abstinence, then EEG treatment to augment these changes is feasible and should be pursued.

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Mechanisms of Neuroplasticity and Ethanol's Effects on Plasticity in the Striatum and Bed Nucleus of the Stria Terminalis

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Long-lasting changes in synaptic function (i.e., synaptic plasticity) have long been thought to contribute to information storage in the nervous system. Although synaptic plasticity mainly has adaptive functions that allow the organism to function in complex environments, it is now clear that certain events or exposure to various substances can produce plasticity that has negative consequences for organisms. Exposure to drugs of abuse, in particular ethanol, is a life experience that can activate or alter synaptic plasticity, often resulting in increased drug seeking and taking and in many cases addiction. Two brain regions subject to alcohol's effects on synaptic plasticity are the striatum and bed nucleus of the stria terminalis (BNST), both of which have key roles in alcohol's actions and control of intake. The specific effects depend on both the brain region analyzed (e.g., specific subregions of the striatum and BNST) and the duration of ethanol exposure (i.e., acute vs. chronic). Plastic changes in synaptic transmission in these two brain regions following prolonged ethanol exposure are thought to contribute to excessive alcohol drinking and relapse to drinking. Understanding the mechanisms underlying this plasticity may lead to new therapies for treatment of these and other aspects of alcohol use disorder.

Key words: Alcohol consumption; ethanol exposure; alcohol use disorder; relapse; brain; neuroplasticity; synaptic function; synaptic plasticity; striatum; stria terminalis; bed nucleus of the stria terminalis

Long-lasting changes in synaptic function (i.e., synaptic plasticity) have long been thought to contribute to information storage in the nervous system (Kandel et al. 2014; Lovinger 2010). Studies combining behavioral and physiological analyses offer strong evidence supporting this hypothesis (Kandel et al. 2014; Ramirez et al. 2014). On the one hand, this plasticity allows the organism to adapt to and function in complex environments; on the other hand, certain events or exposure to various substances can produce plasticity that has negative consequences for the organism (Ursano et al. 2009). Two main types of plasticity are long-term depression (LTD) and long-term potentiation (LTP).

Exposure to drugs of abuse, including beverage alcohol (i.e., ethanol), is one life experience that can activate or alter synaptic plasticity, often resulting in increased drug seeking and taking, and in many cases in addiction (Kauer and Malenka 2007; Luscher and Malenka 2011). Ethanol's actions alter or produce lasting synaptic plasticity in a variety of brain regions, including two regions with key roles in alcohol's actions as well as in control of alcohol intake, namely the striatum and the bed nucleus of the stria terminalis (BNST). These brain regions, in turn, are integral components of several brain circuits, including the cortico-basal ganglia circuits and the extended amygdala. By understanding the types of alcohol-induced synaptic plasticity in these brain regions, we can determine how the drug changes this circuitry. This information will aid in prevention or reversal of such circuit changes in the treatment of alcohol use disorder. The table summarizes the different types of plasticity discussed in this article, as well as the effects of acute and chronic ethanol exposure in these brain regions.

After a brief overview of the cortico-basal ganglia circuits and extended

Table Synaptic Plasticity and Effects of Acute and Chronic Ethanol on Long-Term Potentiation (LTP) and Long-Term Depression (LTD) in the Dorsolateral Striatum (DLS), Dorsomedial Striatum (DMS), Nucleus Accumbens (NAc), and Bed Nucleus of the Stria Terminalis (BNST)

			Acute Ethanol		Chronic Ethanol	
	LTP	**LTD**	**LTP**	**LTD**	**LTP**	**LTD**
DMS/DLS Glutamatergic Synapses	**DMS/DLS** • Activation of N-methyl-D-aspartate (NMDA) receptors (NMDARs) • Insertion of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors (AMPARs) • Stimulation of alpha subunit of the Gs type G protein (Gαs) signaling • Involvement of A2A-type adenosine receptors • Involvement of protein kinase signaling	**DLS** • Decreased probability of vesicle fusion, glutamate release • Endocannabinoid (eCB- mediated inhibition of glutamate release • Activation of dopamine receptor type 2 (D2) dopamine receptors • Activation of meta-botropic glutamate receptor (mGluRs) (groups I and II) • Stimulation of Gi/Go G proteins	**DMS** • Long-term facilitation of glutamatergic transmission • Involves inhibition of NMDAR transmission • Involves stimulation of Fyn tyrosine kinase (TK), phosphorylation of NR2B • Inhibition of NMDAR mediated LTP	**DMS** • Increase in eCB-mediated LTD	**DMS** • Increased LTP • Involves NMDARs (NR2B) and AMPARs • Involves Fyn TK and protein tyrosine phosphatase alpha (PTPα)	**DLS** • Decreased eCB LTD • Secondary to increased 2-AG levels • Loss of mGluR2
DMS/DLS GABAergic Synapses	• Unknown	**DLS** • eCB-mediated LTD at medium spiny neuron (MSN)–MSN and MSN–fast-spiking interneuron (FSI) synapses • Activation of serotonin receptor type 1B (5HT1b) receptors	• Unknown (increased γ-aminobutyric acid [GABA] release in DMS and decreased GABA release in DLS)	• Unknown	• Unknown	• Decreased GABA release in DMS and DLS
NAc Glutamatergic Synapses	• NMDAR activation • AMPAR insertion	• NMDAR-dependent mechanisms (NR2B) • AMPAR removal	• Decreased LTP • Dependent on mGluR (group 1) • Involves altered dopamine release • Decreased LTD • Biphasic, concentration-dependent effect	• Decreased LTD • Restricted to direct pathway MSNs • Involves dopamine receptor type 1 (D1) receptor	• Increased AMPAR function (similar to LTP)	• Decreased LTD in both shell and core • Increased NR2B • Persists for 3 days in shell, recovers after 2 weeks • Decreased tyrosine hydroxylase (TH) and postsynaptic density 95 protein (PSD-95) in shell • Decreased extracellular GluR2
NAc GABAergic Synapses	• Unknown	• Unknown	• Unknown (increased GABAergic transmission)	• Unknown	• Unknown	• Decreased GABA release, altered GABAAR pharmacology & decreased α1 and δ subunits

Table Synaptic Plasticity and Effects of Acute and Chronic Ethanol on Long-Term Potentiation (LTP) and Long-Term Depression (LTD) in the Dorsolateral Striatum (DLS), Dorsomedial Striatum (DMS), Nucleus Accumbens (NAc), and Bed Nucleus of the Stria Terminalis (BNST) (*continued*)

			Acute Ethanol		Chronic Ethanol	
	LTP	**LTD**	**LTP**	**LTD**	**LTP**	**LTD**
BNST Glutamatergic Synapses	• Activation of NMDA receptors (NR2A and NR2B subunits) • LTP subtype mediated by NMDA and mGluR5 receptors	• Dependent on mGluR5, extracellular signal–regulated kinase (ERK) • Involves removal of GluR2 AMPARs from synapse • Mediated by α1 adrenergic receptors • Requires Gq signaling • Due to removal of GluR1 AMPAR from synapse • Involves anandamide and transient receptor potential vanilloid 1 (TRPV1) channels	• Inhibition of LTP • Mediated by NMDAR (NR2B)	• Unknown	• Increased • Increased NR2B • Dampening of LTP in juxtacapsular nucleus	• Decreased • Downregulation of α1A AMPAR

amygdala, this article will discuss ethanol's effects on synaptic plasticity, focusing on the changes produced by ethanol exposure in brain regions that are prominent in the three cortico-basal ganglia circuits. One of the main foci will be on three striatal subregions that have central roles in action control by the three circuits. The article also explores ethanol–plasticity interactions in the BNST, because this brain region has emerged as a prominent player in the drive to obtain drugs of abuse, including ethanol, and also plays a major role in stress–addiction interactions and negative reinforcement.

The many mechanisms that contribute to long-lasting synaptic plasticity have been reviewed extensively in recent years (Atwood et al. 2014; Kandel et al. 2014) and therefore will not be discussed in detail in this review. Similarly, a relatively large literature has described ethanol's effects on synaptic plasticity in other brain regions, especially the hippocampus, that contribute to ethanol-induced cognitive impairment and other aspects of intoxication and the neural effects of chronic alcohol. For example, Zorumski and colleagues (2014) have recently reviewed ethanol's effects on synaptic plasticity throughout the brain and its relationship to altered learning and memory. Therefore, this review will focus on basic mechanisms of synaptic plasticity in BNST and striatum subregions as well as the effects of acute and chronic ethanol exposure on such plasticity. The article concludes with a discussion of the potential contribution of ethanol-induced changes in plasticity in the overall effects of this much-abused drug on the central nervous system, and the potential for interventions that may be developed based on these findings and which eventually may aid in the treatment of alcohol use disorder.

Cortico-Basal Ganglia Loops

A conserved anatomical/physiological motif in the forebrain is the existence of at least three cortico-basal ganglia circuits known as the associative, sensorimotor, and limbic circuits, each of which represents a "loop" connecting the cortex to the basal ganglia and from there back to the cortex (Balleine and O'Doherty 2010; Yin and Knowlton 2006). These circuits help process information about sensory input and internal states as well as generate actions and sequences of actions based on that information. They include the following components:

- The associative circuit consists of associative cortices (e.g., prefrontal cortex and entorhinal cortex), the dorsomedial striatum (DMS) (which corresponds to the caudate nucleus in primates), the downstream basal ganglia subregions, and the thalamus and its projections to the cortex that complete the overall loop structure.

- The limbic circuit connects limbic cortices, including neocortical areas (e.g., medial prefrontal cortex) and "older" cortex (e.g., hippocampus and lateral amygdala), with the ventral striatum (i.e., nucleus accumbens [NAc]) and specific downstream ganglia.

- The sensorimotor circuit includes sensory and motor cortices that project to the dorsolateral striatum (DLS) (which corresponds to the putamen nucleus in primates), with particular basal ganglia and thalamic regions completing this circuit.

Although each circuit likely serves several functions within this overall context, some clearly defined subcircuit functions have emerged (Balleine and O'Doherty 2010; Yin and Knowlton 2006). The associative circuit participates in learning and performing actions based on the outcomes associated with those actions (i.e., goal-directed behavior). This circuit also seems to have a strong role in reward processing (Reynolds et al. 2001; Wickens et al. 2007). The limbic circuit not only integrates information about reward with affective state, but also appears to function prominently in determining the relationship between environmental stimuli and reward. These so-called stimulus–outcome associations contribute to Pavlovian learning and Pavlovian-instrumental transfer (Corbit and Balleine 2011). The sensorimotor circuit features prominently in control of actions by environmental stimuli and perhaps also by internal states. One characteristic of actions controlled by the sensorimotor circuit is that they become less dependent on the expected outcome of an action at any given time and instead are related to the past outcome history (Balleine and O'Doherty 2010; Yin and Knowlton 2006). These sorts of actions are often referred to as habits.

Extended Amygdala

The extended amygdala is a group of structures that includes the amygdala proper, the BNST, and the outer part (i.e., shell) of the NAc. These regions receive input from the prefrontal cortex, thalamus, and hippocampus, usually through connections using the neurotransmitter glutamate, and project to structures in the midbrain, hindbrain, and hypothalamus. This connectivity suggests that the extended amygdala can act as a means to coordinate broad behavioral states. This anatomical construct, and in particular the central nucleus of the amygdala and the BNST, has received much attention for its role in the regulation of negative reinforcement. Briefly, dysregulation of activity in the central nucleus of the amygdala is thought to alter output to the BNST. The BNST, in turn, can then regulate stress responses by activating the body's hormonal stress response system (i.e., the hypothalamic–pituitary–adrenal axis), as well as reward behavior by acting on regions called the ventral tegmental area and dorsal raphe nucleus. In general, the extended amygdala does not seem to directly influence functions in the dorsal striatum. However, because neurotransmitters involved in stress responses and reward behaviors (e.g., corticosterone, dopamine, and serotonin) can influence striatal plasticity, a functional link clearly exists between the extended amygdala and the striatum.

Subregions within the extended amygdala either are part of the limbic cortico-basal ganglia circuit or interact heavily with the main regions within this circuit. In this context, the BNST is of particular interest, because it not only influences striatal function but is also related to addiction and responsivity to alcohol as well as to relapse. In addition, the BNST may be part of a "neuroendocrine" or "interoceptive" basal ganglia circuit (Dong and Swanson 2003, 2004; Dong et al. 2001*a,b*) and therefore also fits into the general circuitry system described above.

A growing body of literature suggests the involvement of BNST in addiction. As part of the central extended amygdala this brain region is extensively interconnected with hypothalamic, midbrain, and hindbrain regions (Walker et al. 2003). In addition to the complex inputs to and outputs from the BNST, the structure itself comprises multiple subregions and cell types, the details of which are only now emerging (for a review, see Lowery-Gionta and Kash 2014). The BNST is altered, either functionally or structurally, by a variety of by a variety of drugs, including morphine, cocaine, heroin, and ethanol, and is critical for stress-related reinstatement of drug-seeking behavior (for a review, see Lowery-Gionta and Kash 2014). The BNST also is essential for alcohol–withdrawal-induced anxiety, conceptually supporting the hypothesis that the BNST regulates relapse to ethanol, and use (Huang et al. 2010). Other studies have demonstrated the involvement of the BNST in the modulation of stress- and anxiety-related behaviors (Walker et al. 2009). Given that stress and anxiety may be essential in shaping alcohol-related behavioral pathology as well as the connectivity of the BNST to mid- and hindbrain regions that can broadly influence the brain, understanding how both acute and chronic alcohol exposure can regulate plasticity in the BNST is crucial.

Striatal Synaptic Plasticity

LTP

Various types of activity-dependent synaptic plasticity have been observed in the dorsal and ventral striatum, including LTP and LTD. LTP is a process leading to long-lasting enhancement of signal transmission between two neurons that occurs when the two neurons are stimulated repeatedly at the same time. Similarly, LTD refers to a process by which signal transmission between two cells decreases after repeated stimulation. Both of these processes are thought to contribute to memory and learning. LTP occurs, for example, at glutamatergic synapses in the striatum. This process seems to involve mechanisms very similar to those implicated in the best- characterized LTP subtypes that occur at hippocampal synapses (Bliss and Collingridge 2013; Calabresi et al. 2007; Gerfen and Surmeier 2011). Induction of LTP in the striatum begins with activation of certain postsynaptic receptors for the neurotransmitter glutamate (i.e., the N-methyl-D-aspartate [NMDA] receptors [NMDARs]). Activation of these receptors ultimately results in an increase in transmission, most likely through the insertion of another type of glutamate receptor (i.e., alpha-amino-3-hydroxy-5-

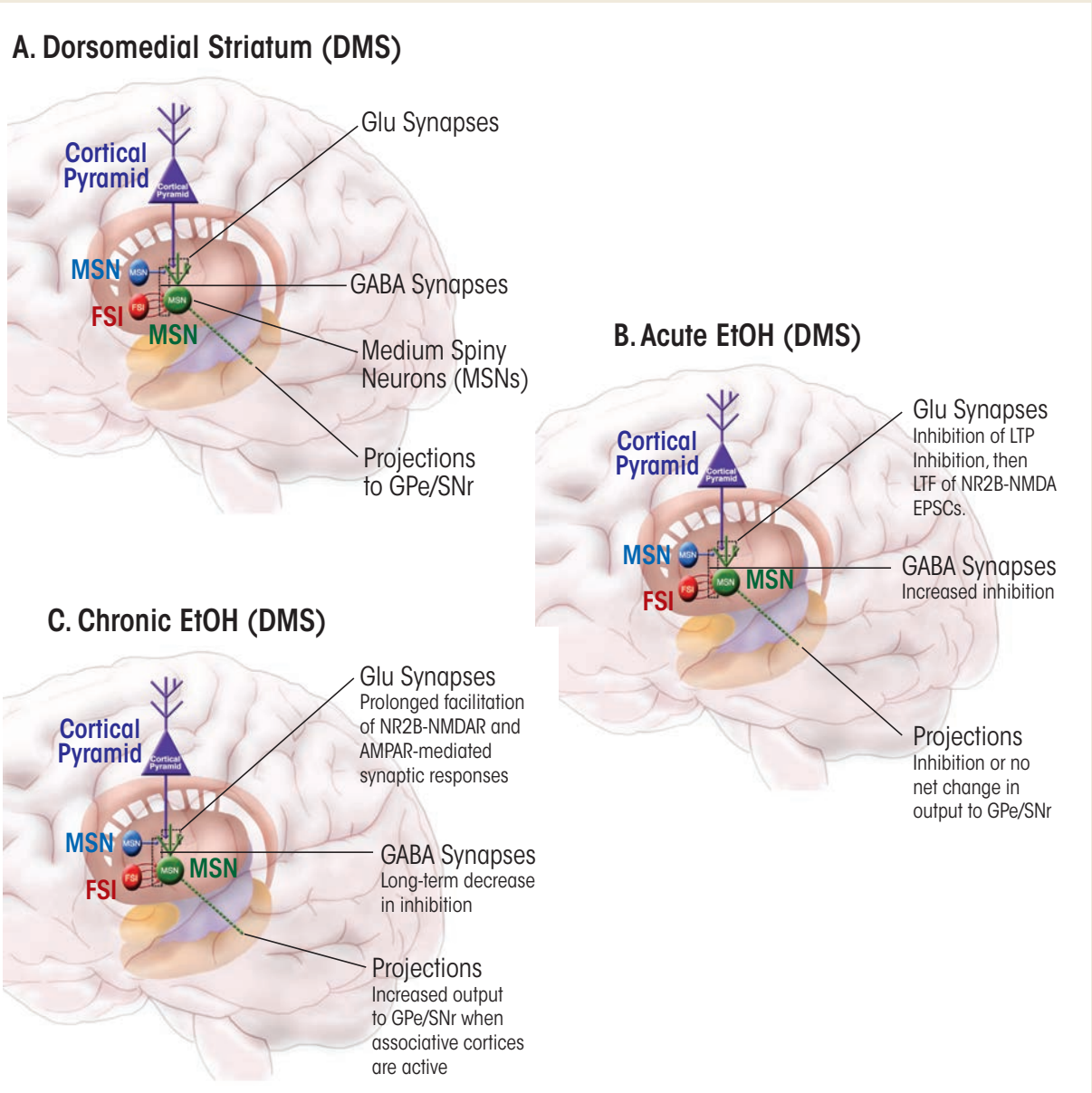

Figure 1 Schematic illustration of neuronal circuits in the dorsomedial striatum (DMS) and of the effects of acute and chronic ethanol exposure on plasticity in this region. **(A)** Simplified diagram of the circuits in the DMS, showing glutamatergic cortical inputs to the major projection neurons in the striatum (i.e., medium spiny neurons [MSNs]). Also indicated is GABAergic microcircuitry involving MSN–MSN synapses that tend to innervate dendrites and synapses made by fast-spiking interneurons (FSIs) on MSN cell bodies. These MSNs project out of the striatum to the globus pallidus external segement (GPe) and the substantia nigra pars reticulata (SNr). Boxed areas indicate the predominate sites of synapses on the MSNs. **(B)** Effects of acute ethanol exposure on plasticity at synapses onto DMS MSNs. The net effects are prevention of normal plasticity (i.e., inhibition of long-term potentiation [LTP]) at excitatory cortical glutamatergic inputs, while a new form of NMDA receptor (NMDAR)-dependent long-term facilitation (LTF) occurs. Increased synaptic inhibition also occurs. Thus, the net signal output from the DMS may be dampened, while responses to associative cortical input may become aberrant. **(C)** Effects of chronic ethanol exposure on plasticity at synapses in the DMS. Net effects include prolonged LTF and LTP-like increase in AMPA receptor function at glutamatergic synapses, accompanied by net decreases in inhibition. These changes may alter goal-directed ethanol-related behaviors, particularly those controlled by the prefrontal cortex and related associative cortices.

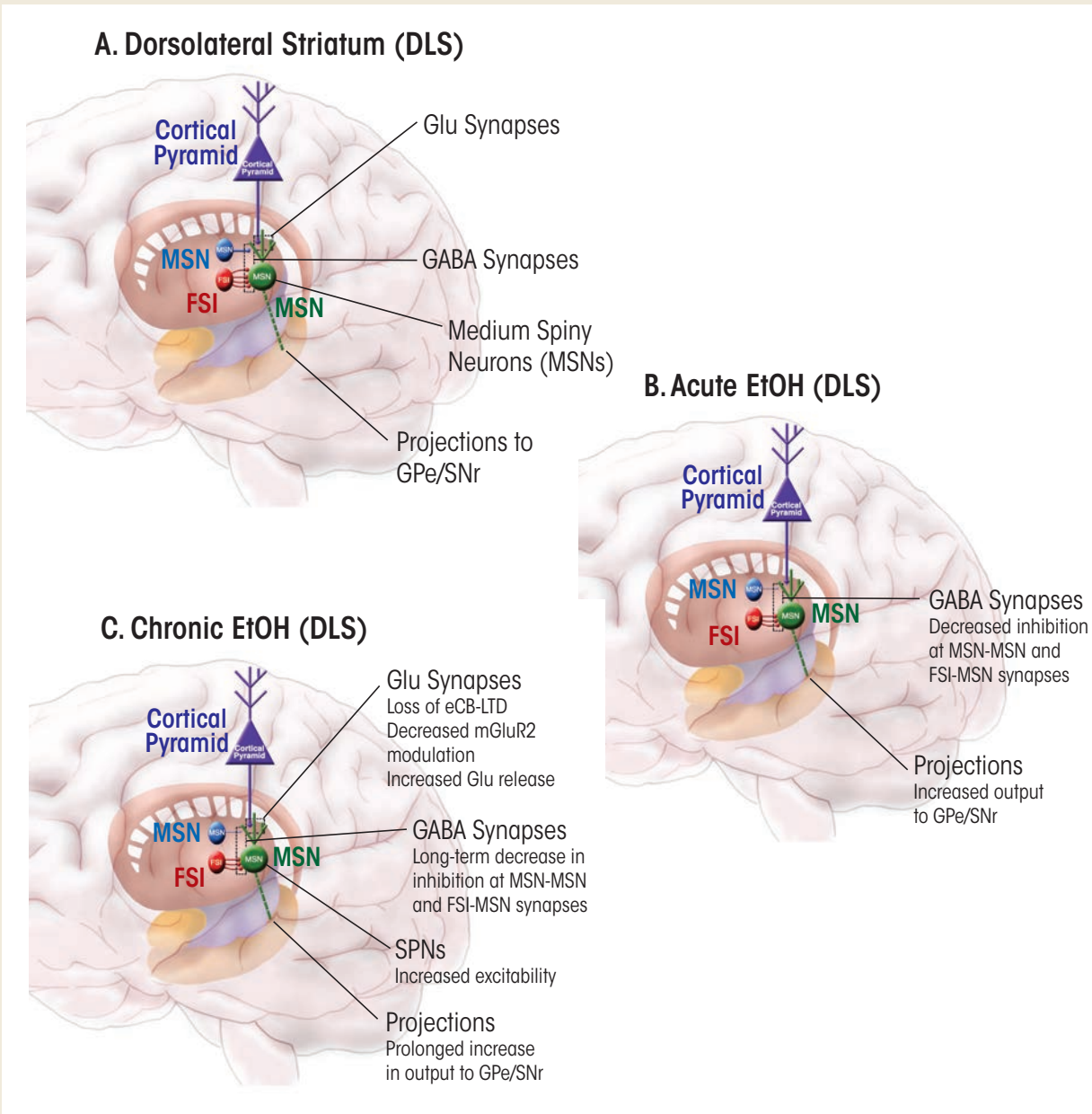

Figure 2 Schematic illustration of neuronal circuits in the dorsolateral striatum (DLS) and of the effects of acute and chronic ethanol exposure on plasticity in this region. **(A)** Simplified diagram of the circuits in the DLS, showing glutamatergic cortical inputs to the major projection neurons in the striatum (i.e., medium spiny neurons [MSNs]). Also indicated is GABAergic microcircuitry involving MSN–MSN synapses that tend to innervate dendrites and synapses made by fast-spiking interneurons (FSIs) on MSN cell bodies. These MSNs project out of the striatum to the globus pallidus external segement (GPe) and the substantia nigra pars reticulata (SNr). Boxed areas indicate the predominate sites of synapses on the MSNs. **(B)** Effects of acute ethanol exposure on plasticity at synapses onto DLS MSNs. The major net effect described to date is decreased inhibition, which would increase net output from sensorimotor striatum and perhaps initiate habit formation. **(C)** Effects of chronic ethanol exposure on plasticity at synapses in the DLS. The net effects are decreased presynaptic endocannabinoid (eCB)-dependent long-term depression (LTD), increased MSN excitability, and decreased inhibitory GABAergic transmission onto MSN. These changes should foster greater DLS output in response to a given set of inputs from sensorimotor cortex, potentially facilitating habit formation.

methyl-4-isoxazolepropionic acid [AMPA] receptors [AMPARs]) into the synaptic plasma membrane (Gerfen and Surmeier 2011). Striatal glutamatergic LTP is not as well characterized as is hippocampal LTP; however, it is known to have a few unique features—for example, regarding the signaling cascades (i.e., G-protein signaling) that are induced during LTP. Thus, for striatal LTP, the activation of G-protein–coupled receptors that stimulate a certain type of G-protein (i.e., the GαS type of G-protein) seems to be a key step. The majority of experiments indicate that activation of D1 dopamine receptors also is a crucial step in striatal LTP; however, these receptors are only expressed on a certain cell type (i.e., direct pathway medium spiny projection neurons [MSNs]) that make up about 45 percent of all striatal neurons (Calabresi et al. 2007). In the other striatal neurons, different classes of Gs-coupled receptors may be involved, and indeed the A2A-type adenosine receptor has been implicated in LTP induction (Gerfen and Surmeier 2011). Activation of other signaling pathways (i.e., protein kinases) also may contribute to striatal LTP.

LTD

The major form of LTD at striatal glutamatergic synapses involves a decrease in the probability that vesicles containing the neurotransmitter fuse with the membrane of the signal-emitting (i.e., presynaptic) neuron and release their glutamate, thereby initiating signal transmission (Atwood et al. 2014). The best-characterized form of this LTD involves release by the signal-receiving (i.e., postsynaptic) cell of an endocannabinoid (eCB) that then acts back on the presynaptic cell, where it activates presynaptic CB1 receptors, resulting in inhibition of glutamate release. Induction of this form of LTD requires the activation of D2 dopamine receptors and a glutamate receptor subtype (i.e., group I mGluR receptors). Numerous other neuromodulators also can initiate LTD at striatal synapses, including activation of certain serotonin receptors (i.e., 5-HT1b receptors) and another group of glutamate receptors (i.e., mGluR2 receptors) (Atwood et al. 2014). Despite their diversity, these receptors all couple to Gi/o-type G-proteins and produce long-lasting suppression of neurotransmitter release from the presynaptic cell.

Other forms of synaptic plasticity also have been described at glutamatergic synapses in striatum. These include NMDAR-dependent depotentiation of glutamatergic transmission onto MSNs (Calabresi et al. 2007), as well as plasticity of glutamatergic transmission onto non-MSN striatal neurons.

In addition to glutamatergic synapses, those that use γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, also exhibit both LTP and LTD (McBain and Kauer 2009; Nugent and Kauer 2008). For example, eCB-mediated presynaptic LTD has been described at GABAergic synapses onto MSNs in the striatum (Adermark and Lovinger 2009; Mathur et al. 2013). There seem to be two subtypes of eCB-dependent LTD at GABAergic synapses in this region, depending on the types of cells forming the synapses. Thus, one subtype of eCB-dependent LTD affects MSN–MSN synapses and another affects synapses transmitting signals from fast-spiking parvalbumin-expressing interneurons (FSIs) to MSNs. Activation of 5-HT1b receptors also produces LTD at striatal GABAergic synapses, and the mechanisms of this presynaptic form of LTD appear to overlap with those of eCB-mediated LTD (Mathur et al. 2011). More work is needed to determine what other forms of synaptic plasticity occur at these GABAergic synapses, because control over this inhibitory neurotransmitter presents a powerful tool to regulate striatal output, which could strongly influence action selection and responses to drugs of abuse.

BNST Synaptic Plasticity

LTP

Similar to the striatum, both LTP and LTD have been found in the BNST. Thus, Weitlauf and colleagues (2004) determined that extended stimulation in the dorsal lateral BNST could support LTP and that this process was dependent on NMDAR signaling. At the time, NMDAR-dependent LTP was thought to require signaling via the GluN2A receptor subunit (Liu et al. 2004), based on studies using a new compound called NVP-AAM077 that was purported to selectively block the GluN2A subunit. Consistent with previous studies, NVP-AAM077 blocked LTP in the BNST, suggesting the involvement of GluN2A (Weitlauf et al. 2005). However, both LTP and the inhibitory effect of NVP-AAM077 persisted in mice that did not carry any GluN2A receptors. Subsequent mechanistic studies found that under certain conditions NVP-AAM077 also could inhibit NMDARs containing the GluN2B receptor subunit, suggesting that the selectivity of this compound may not be as strong (Frizelle et al. 2006). More recently, Wills and colleagues (2012) used an experimental approach that could control activation and inactivation of the GluN2B subunit to demonstrate that this form of LTP is critically dependent on the presence of GluN2B-containing NMDARs.

In addition to this form of LTP, other investigators identified a novel type of LTP of intrinsic excitability that occurred in the juxtacapsular nucleus of the BNST after a high-frequency stimulation and which could be prevented by inhibition of both NMDA and mGluR5 receptors (Francesconi et al. 2009*a,b*). This type of plasticity may be a homeostatic mechanism to prevent excessive anxiety-like behaviors, because the juxtacapsular nucleus has been proposed to have a feedback inhibitory input

to the basolateral amygdala, which in turn control fear responses.

LTD

Several forms of LTD can be expressed in the BNST. Grueter and colleagues (2006) identified a mGluR5-mediated form of LTD induced by activation of group 1 mGluR receptors. This particular plasticity was cannabinoid independent but required extracellular signal-regulated protein kinase (ERK). Moreover, this LTD was found to affect the postsynaptic rather than presynaptic cell and involved removal of GluR2-containing AMPA receptors from the synapse (Grueter et al 2008). A similar form of plasticity is mediated by alpha1 adrenergic receptors (α1-ARs) (McElligott and Winder 2008). At first glance, these two forms of LTD appear to be the same, because they both are mediated via Gq signaling and appear to result from postsynaptic AMPA receptor trafficking. However, the α1-AR mediated LTD results from removal of GluR1 subunits rather than GluR2 subunits from the AMPA receptors (McElligott et al. 2010). Finally, other investigators identified a third type of LTD in the BNST that was dependent on mGluR5-mediated generation of the lipid signal anandamide, which then acted on postsynaptic transient receptor potential V1 (TRPV1) channels (Puente et al. 2011). Although this type of LTD was not directly compared with the LTD identified by Grueter and colleagues (2006), it is likely that they are similar.

Acute Ethanol and Striatal Synaptic Plasticity

Striatal Glutamatergic Synapses

Acute intoxication occurs over a range of brain ethanol concentrations from approximately 5 to 100 mM, with increasing severity as the concentration ascends. Thus far, only a few studies have examined the acute effects of ethanol on plasticity. One series of studies using brain slices of the DMS found that a lasting facilitation of glutamatergic transmission mediated by NMDARs occurred after the slices had been exposed for a few minutes t o 25 to 100 mM ethanol (Wang et al. 2007, 2010, 2012). The investigators dubbed this process long-term facilitation (LTF) to distinguish it from LTP, because LTF involves a lasting increase in NMDAR function and may not share all of the mechanisms involved in LTP. LTF occur only after the inhibition of NMDAR-mediated transmission, normally observed during acute ethanol exposure, ends (see figure 1A and 1B). It is thought that ethanol stimulates Fyn tyrosine kinase, which then mediates phosphorylation of the NR2B NMDAR subunit, thereby inducing LTF (Gibb et al. 2011; Wang et al. 2010). Indeed, LTF only affects transmission mediated by receptors that contain NR2B.

It has long been postulated that ethanol-induced alterations in synaptic function underlie many of the drug's neuroadaptive effects that contribute to tolerance, physical dependence, and addiction.

In addition to inducing LTF, acute ethanol exposure inhibits the induction of NMDAR-mediated LTP in the DMS (Yin et al. 2007) (figure 1B). Reductions in LTP magnitude, which can be observed at ethanol concentrations at the low end of the intoxicating range, most likely involve other mechanisms in addition to inhibition of NMDARs and perhaps also phosphorylation mediated by ERK. This reduction in LTP magnitude is accompanied by an increase in the magnitude of eCB-mediated LTD in DMS.

Acute ethanol exposure inhibits both LTP and LTD in the NAc/ventral striatum (Jeanes et al. 2011; Mishra et al. 2012). Thus, ethanol prevents the induction of LTP by high-frequency stimulation in this brain region (Mishra et al. 2012). This effect may involve both inhibition of responses to group I mGluRs as well as altered dopamine release. Other studies have shown that acute ethanol also alters an NMDAR-dependent form of LTD in the NAc, and particularly in the NAc shell (Jeanes et al. 2011). Normally, sustained afferent stimulation at low to moderate frequencies induces an NMDAR-dependent form of LTD in this striatal subregion (Jeanes et al. 2011; Thomas et al. 2000), which can be prevented by antagonists of NMDARs that contain the NR2B subunit. Acute ethanol exposure has a biphasic concentration-dependent effect on this NAc-LTD, with complete inhibition observed at 40 mM ethanol, but less effect at concentrations of 20 mM and 60 mM. Further analyses demonstrated that ethanol's effect on this form of plasticity is restricted to certain cells—namely, MSNs that are part of the "direct" output pathway (Jeanes et al. 2014). In contrast to other MSNs, these cells express D1 dopamine receptors. The fact that this effect exclusively occurs in these MSNs is consistent with findings indicating that D1 receptors are involved in this form of LTD and that ethanol and D1 receptors seem to have antagonistic effects in preventing and restoring LTD (Jeanes et al. 2011). Previous work showing that D1 receptor activation counteracts ethanol-induced inhibition of NMDARs indicates a likely mechanism for this interaction.

Striatal GABAergic Synapses

No studies have explicitly analyzed the effects of acute ethanol exposure on LTP or LTD at striatal GABAergic synapses. It is known that acute ethanol increases GABAergic transmission in the DMS, while producing the

opposite effect (i.e., inhibition) at synapses in the DLS (Wilcox et al. 2014) (figure 1B and 2B). It is tempting to speculate that the increased GABA-mediated inhibition in the DMS and disinhibition in the DLS favor increased function of the sensorimotor circuit; the implications of such a scenario will be discussed later in this review.

The effects of acute ethanol exposure on GABAergic synapses in the NAc/ventral striatum also have not been examined in great detail. Nie and colleagues (2011) reported that ethanol potentiated responses to applied GABA in a subset of MSNs in the NAc core. Furthermore, Mishra and Chergui (2013) offered evidence that increased GABAergic transmission in the NAc underlies the inhibitory effects of ethanol. However, neither of these studies directly measured the effects of acute ethanol on GABA-mediated synaptic responses. A recent study indicated that acute ethanol potentiates tonic currents in the NAc that are mediated by $GABA_A$ receptors (Liang et al. 2014*b*). This effect seems to be attributable to increased function of the $GABA_A$ receptors, although increased GABA release also could play a role. Given the large body of literature implicating the NAc in controlling ethanol intake (Anstee et al. 2013; Hodge et al. 1995; Hyytia and Koob 1995; June et al. 1998; Nie et al. 2011; Rewal et al. 2009), additional characterization of ethanol's effects on GABAergic transmission in this brain region certainly is warranted.

Acute Ethanol and Synaptic Plasticity in the BNST

A few studies have examined the impact of acute alcohol on synaptic plasticity in the BNST. An initial study by Weitlauf and colleagues (2004) found that application of ethanol to a brain slice inhibited induction of LTP in the BNST. Interestingly, this impairment was limited to the early phase of LTP. Alcohol's effect seemed to be mediated via actions on the NMDAR, because the alcohol concentration employed inhibited NMDARs but did not alter $GABA_A$-mediated currents. A subsequent analysis revealed that acute alcohol specifically inhibited NMDAR-mediated but not AMPAR-mediated currents in the BNST (Kash et al. 2008*a*). In addition, this study used a pharmacological approach to determine if specific subtypes of NMDARs were involved in this inhibition. These analyses found that the presence of an NR2B-selective antagonist, Ro 25-2981, prevented ethanol's inhibitory effect, suggesting that ethanol selectively targets NR2B-containing NMDARs in the BNST to exert its inhibitory effect on LTP. This model was supported by an elegant study demonstrating that genetic inactivation of NR2B could remove the alcohol sensitivity of NMDAR-mediated responses (Wills et al. 2012).

The lack of ethanol effects on GABAergic transmission in the BNST is intriguing, especially because this structure is similar to the central nucleus of the amygdala, where ethanol robustly increases GABA release (Gilpin et al. 2014). Furthermore, Wills and colleagues (2013) confirmed that ethanol had no effect on GABA transmission in the BNST in adult animals, but could enhance GABA transmission in adolescent animals. This developmental control of sensitivity to ethanol is interesting, because it suggests that alcohol modulation of plasticity and circuitry is dynamic. Considering the connectivity of the BNST and its role in the regulation of anxiety-like behavior and arousal states, it is tempting to speculate that ethanol inhibition of LTP in this region is linked to the anxiolytic actions of alcohol.

Effects of Chronic Ethanol on Synaptic Plasticity at Striatal Synapses

Striatal Glutamatergic Synapses

It has long been postulated that ethanol-induced alterations in synaptic function underlie many of the drug's neuroadaptive effects that contribute to tolerance, physical dependence, and addiction (Lovinger and Roberto 2013; Vengeliene et al. 2008; Zorumski et al. 2014). Most research on this topic has focused on brain regions other than striatum. In recent years, however, a number of groups have begun to examine how striatal synapses are altered during and following chronic ethanol exposure. From these studies, interesting patterns are emerging that indicate how production of aberrant communication at synapses in corticostriatal circuits and striatal microcircuits contributes to alterations in responses to alcohol-associated cues, the rewarding properties of ethanol, and habitual alcohol-seeking/drinking behavior that are characteristic of alcohol use disorder.

Effects on LTD

The eCB-dependent striatal LTD discussed earlier may be important in forms of learning and memory that involve this brain region (DePoy et al. 2013; Hilario et al. 2007; Yin et al. 2009). In particular, Hilario and colleagues (2007) found that CB1 receptors are key contributors in "habitual" learning and performance of instrumental actions (i.e., actions that are relatively insensitive to reward contingency). This type of learning involves the dorsal striatum, and in particular the sensorimotor striatum or DLS. Thus, it is possible that the promotion of habitual alcohol-seeking after repeated exposure to the drug (Barker et al. 2010; Corbit et al. 2012; Dickinson et al. 2002; Mangieri et al. 2012) involves changes in striatal eCB-mediated LTD. Indeed, Xia and colleagues (2006) found that this type of LTD decreased in magnitude in animals administered ethanol for 10 days, with the decrease persisting after ethanol withdrawal (figure 2C). Loss of eCB-LTD in the DLS also was observed in brain slices obtained from animals that had been exposed to ethanol using an inhalational model

(DePoy et al. 2013). This loss was accompanied by an increase in striatal levels of an eCB called 2-arachidonoyl glycerol (2-AG). It is possible that unnaturally high levels of this eCB produce excessive LTD in the in vivo state. This in vivo LTD may occlude subsequent induction of LTD in slices. Alternatively, the high 2-AG levels could alter signaling mechanisms necessary for LTD induction in the brain slices (e.g., by causing reductions in the number of receptors or impaired signaling). It is tempting to speculate that this loss of plasticity contributes to ethanol effects that promote habitual drug seeking, but experiments to test this hypothesis have not yet been carried out.

The eCB-mediated LTD is just one form of presynaptically expressed LTD that is initiated by the activation of Gi/o-coupled receptors (Atwood et al. 2014). Glutamatergic synapses in the striatum (including those on MSNs in both dorsal and ventral striatum) appear to express presynaptic LTD driven by several receptor subtypes. For example, activation of presynaptic group II mGluRs (which includes mGluRs 2 and 3) can induce LTD in the striatum. Recent studies indicate that mGluR2 is the main receptor subtype involved in this form of LTD and that loss of this receptor is associated with higher ethanol intake in rats and mice (Meinhardt et al. 2013; Zhou et al. 2013). Indeed, in the widely studied ethanol-preferring (P) rats developed by selective breeding, the gene encoding mGluR2 receptors is defective and causes premature termination of the receptor (Zhou et al. 2013).

Effects on LTP

LTP also is thought to have key roles in learning and memory in a variety of brain regions (Kandel et al. 2014), including the striatum (Dang et al. 2006; Lovinger 2010; Reynolds et al. 2001). Chronic exposure to drugs of abuse may alter or engage pivotal synaptic plasticity mechanisms to produce learning and memories related to the drugs and associated environmental events. Thus, the effects of drugs on LTP have been widely studied, and it is clear that chronic ethanol exposure alters LTP in brain regions such as the hippocampus (for a review, see Zorumski et al. 2014).

In the striatum, the net effect of chronic ethanol has been less well studied, but the work to date indicates that ethanol exposure brings about an LTP-like enhancement of synaptic efficacy at glutamatergic synapses that transmit signals to striatal MSNs. As mentioned previously, Wang and colleagues (2010) have described an increase in NMDAR-mediated synaptic transmission that begins just after the end of an acute exposure to ethanol and which seems to be largest in the DMS (figure 1C). Because the AMPAR-mediated component of glutamatergic transmission is unaltered at that stage, the observed LTF seems to be specific to NMDARs (Wang et al. 2010). However, given the key role of NMDARs in the LTP induction process, increasing NMDAR function might be expected to increase the likelihood of induction of an LTP-like form of plasticity that would involve increased AMPAR-mediated transmission (Collingridge 2003). Indeed, further studies demonstrated that either repeated acute exposure of brain slices to ethanol or repeated in vivo ethanol exposure enhanced the ability to induce LTP in the DMS (Wang et al. 2010). Furthermore, chronic ethanol exposure enhanced not only NMDAR-mediated transmission onto DMS MSNs (Wang et al. 2010) but also synaptic AMPAR expression (Wang et al. 2012). Thus, chronic exposure to ethanol gradually sets up conditions that favor induction of LTP-like increases in glutamatergic efficacy in striatum, perhaps contributing to alterations in ethanol intake or environmental control of intake.

Indeed, LTF of NMDARs and the potentiation of AMPAR-mediated transmission both seem to contribute to altered ethanol intake. Thus, injection into the DMS (but not into the DLS) of an antagonist for NR2B-containing NMDARs reduced operant responding for ethanol (Wang et al. 2010). Antagonist injection into the DMS also decreased ethanol-primed increases in operant responding for the drug. Similarly, injection of AMPAR antagonists into the DMS decreased operant responding for ethanol (Wang et al. 2012). Further analyses have implicated a signaling pathway involving changes in phosphorylation of the NR2B subunit that involves the Fyn tyrosine kinase (Gibb et al. 2011; Wang et al. 2010). Additional evidence suggests that manipulations of the activity of this kinase as well as of protein tyrosine phosphatase α (PTPα) in the DMS alter ethanol drinking and preference (Ben Hamida et al. 2013).

Effects on LTD and LTP in the NAc

Effects of chronic ethanol exposure on LTD and LTP of glutamatergic transmission have also been examined in the NAc shell of mice (Jeanes et al. 2011). The NAc LTD described earlier, which depended on the NR2B receptor subunit, disappears if plasticity is assessed 1 day after a relatively short inhalational exposure to chronic ethanol; instead, this stimulation protocol induces an NMDAR-dependent form of LTP. The NR2B receptor subunit is altered by both acute and chronic ethanol exposure (Carpenter-Hyland et al. 2004; Floyd et al. 2003; Wang et al. 2010, 2012), suggesting that changes in the expression or function of this subunit contribute to ethanol-induced changes in plasticity. Indeed, increased expression of the NR2B subunit occurs in the NAc following chronic ethanol exposure (Obara et al. 2009; Szumlinski et al. 2008). The decrease in LTD induced by chronic ethanol persists for 3 days, but is fully reversed 2 weeks after the end of ethanol exposure. The ethanol-exposure regimen that altered NAc-LTD also produced increased ethanol intake in mice. Thus, it is tempting to think that the changes in synaptic efficacy at NAc-shell synapses contribute

to the change in intake; however, more work will be needed to confirm this hypothesis.

Other investigators recently also demonstrated decreased LTD in the NAc shell during withdrawal following chronic ethanol exposure in rats (Spiga et al. 2014). Alterations in expression of several proteins (i.e., tyrosine hydroxylase, PSD-95 postsynaptic density protein), alterations in dendritic spine morphology, and decreased NMDAR-mediated synaptic transmission all accompanied the loss of LTD. Thus, the array of molecular changes accompanying and possibly contributing to chronic ethanol-induced loss of NAc-shell LTD is expanding.

Decreased NMDAR-dependent LTD also has been observed in the NAc-core following chronic ethanol exposure and is associated with locomotor sensitization (Abrahao et al. 2013). Indeed, LTD was normal in mice that did not exhibit sensitization following repeated ethanol injections, but decreased in magnitude in those animals that exhibited sensitization following the chronic drug exposure regimen. The loss of NAc-core LTD likely was caused by decreased NMDAR-mediated synaptic responses, which accompanied this form of plasticity. It is interesting to note that mice that showed sensitization also exhibited increased ethanol intake in a drinking-in-the-dark paradigm. Thus, these findings reinforce the idea that loss of NAc LTD is associated with increased ethanol intake.

Another intriguing study has linked NMDARs with the development of ethanol drinking that does not decrease (as occurs normally) when it results in aversive consequences. Specifically, this aversion-resistant drinking was associated with increased expression of NMDARs that contain the NR2C subunit at synapses where glutamatergic cells from the medial prefrontal cortex and insular cortex connect with NAc MSNs (Seif et al. 2013). Transmission mediated by these receptors is more prominent at hyperpolarizing potentials compared with transmission mediated by receptors containing other NR2-type subunits. Furthermore, inhibition of the NR2C-containing receptors in the NAc core reduced the aversion-resistant drinking. This finding indicates that an increased contribution of NMDARs to synaptic transmission may help to drive drinking under stressful/aversive conditions via actions in the limbic circuitry.

However, it is not only changes in postsynaptic NMDAR-mediated synaptic transmission in the NAc that seem to contribute to chronic ethanol effects and alcohol-related behaviors; alterations in AMPAR-mediated synaptic transmission also seem to play a role. Thus, prolonged chronic ethanol exposure was associated with an increase in AMPAR-mediated transmission (Marty and Spigelman 2012). This increase resulted from enhancement of synaptic AMPARs lacking the GluA2 subunit, a change that has also been observed following LTP induction (Isaac et al. 2007), reinforcing the idea that prolonged ethanol exposure can induce LTP-like increases in glutamatergic transmission.

Effects on Extracellular Glutamate

Chronic ethanol exposure also may affect extracellular glutamate levels in various brain regions, including the NAc (Gass et al. 2011; Melendez et al. 2005; Szumlinski et al. 2007, 2008). However, it is not clear if these changes involve altered synaptic glutamate release or result from increased glutamate coming from neuronal transporters or other cellular sources (for a review, see Marty and Spigelman 2012). Interestingly, prolonged chronic-intermittent ethanol exposure is associated with decreased expression of presynaptic mGluR2 by neurons in the infralimbic cortex, a prominent region in the limbic circuit. These infralimbic cortex neurons project to the NAc where they release glutamate from their presynaptic terminals. The mGluR2 on these terminals helps control glutamate levels by limiting glutamate release through autoreceptor feedback (Meinhardt et al. 2013). Accordingly, decreased function of this receptor could contribute to the increased extracellular glutamate levels associated with elevated intake following chronic exposure. Other studies demonstrated that restoring receptor expression in the infralimbic cortex reduced the elevated drinking observed in the chronically exposed animals (Meinhardt et al. 2013). These findings, along with studies showing that alcohol-preferring P rats lack mGluR2 (Zhou et al. 2013), indicate that a decrease or loss of this receptor leads to insufficient control of glutamate release, which ultimately may contribute to excessive alcohol drinking.

Striatal GABAergic Synapses

In addition to the documented alterations in glutamatergic transmission induced by chronic alcohol, evidence indicates that chronic ethanol exposure also induces changes in GABAergic synaptic transmission in striatal microcircuits. These forms of plasticity also may contribute to increased ethanol seeking and intake.

In the dorsal striatum, GABAergic transmission is decreased following chronic ethanol drinking. For example, GABAergic transmission declined in both the DMS and DLS of mice who had been consuming ethanol for 6 weeks under a drinking-in-the-dark regimen (Wilcox et al. 2014) (figure 1C and 2C). A similar decrease in GABAergic transmission was observed in the putamen of Cynomolgus macaque monkeys, which is roughly equivalent to mouse DLS (Cuzon Carlson et al. 2011). The reasons underlying this decrease in transmission remain to be determined. Analyses of miniature inhibitory postsynaptic currents (mIPSCs) in striatal neurons from these mice and monkeys suggested possible synaptic loci underlying the chronic ethanol-induced decrease in GABAergic transmission. The most consistent finding was a decrease in mIPSC frequency, indicating either a decrease in GABA release or a decreased

number of GABAergic synapses on MSNs in the sensorimotor striatum.

Liang and colleagues (2014*a*) have examined the effects of prolonged intragastric ethanol exposure on the properties and pharmacology of GABAergic synapses and tonic $GABA_A$ receptor-mediated transmission onto NAc MSNs. The major changes were in receptor pharmacology, with decreased potentiation in response to acute ethanol and diazepam and increased effects of a compound called RO15-4513. This compound partially inhibits the receptor through an action known as partial inverse agonism. These changes certainly could contribute to tolerance to the CNS effects of both ethanol and sedative benzodiazepines. Chronic ethanol also decreased the amplitude and frequency while increasing the rise time of GABAergic mIPSCs, indicating postsynaptic changes at GABAergic synapses. Furthermore, the NAcs of mice chronically exposed to ethanol exhibited changes in cell surface levels of several $GABA_A$ receptor subunits, including decreased expression of alpha1 and delta subunits and increased expression of alpha4 and alpha5 subunits. Some of these changes may well contribute to the postsynaptic changes at GABAergic synapses. Surprisingly, the tonic $GABA_A$ receptor-mediated current was not altered in these neurons, despite the decreased expression of the delta receptor subunit that mediates this current. Similarly, chronic ethanol exposure did not alter dopamine modulation of the tonic $GABA_A$ receptor- mediated current (Liang et al. 2014*b*). Finally, chronic ethanol exposure led to decreased frequency of mIPSCs, which seemed to result mainly from a decrease in occurrence of mIPSCs with fast rise times. This may reflect a decrease in GABA release or in the number of synapses at a particular input to these MSNs.

Striatal Synaptic Plasticity and Alcohol Seeking/Intake

When evaluating alcohol-induced changes in synaptic plasticity and alcohol-related behaviors, it is important to consider the pattern and duration of ethanol exposure. Most of the chronic exposure paradigms discussed here involve periods of ethanol availability alternating with periods of forced withdrawal or abstinence. Synaptic and behavioral changes brought about by ethanol exposure alone should be carefully compared with changes only observed when exposure and withdrawal/abstinence occur, because the in vivo outcomes differ with these different paradigms (Becker and Hale 1993; Lopez and Becker 2005).

The contributions of the various types of ethanol-induced striatal synaptic changes to the neuroadaptation and behavioral changes associated with excessive alcohol intake and alcohol dependence are the subject of considerable ongoing investigation. A prominent role for the dorsal striatum in the control of alcohol intake is just starting to emerge. Thus, it appears that the associative striatum contributes to alcohol seeking and intake at stages where these behaviors still are under the control of "goal-directed" strategies. However, when seeking and taking become more habitual (i.e., less dependent on the outcome following a behavior), the contributions of sensorimotor striatal regions may become more prominent. Although this scenario is supported by behavioral evidence, little is known about the molecular, synaptic, and cellular mechanisms that contribute to the different alcohol-seeking and -taking strategies. It will be interesting to determine how the mechanisms described above contribute to goal-directed and habitual alcohol seeking and drinking.

By contrast, many studies have critically implicated the NAc in controlling intake of alcohol and other drugs of abuse (Belin et al. 2009; Koob 2013; Marty and Spigelman 2012). The ethanol-induced synaptic alterations and changes in synaptic plasticity in that brain region have been postulated to contribute to the excessive alcohol intake associated with alcohol use disorder. Strong tests of this hypothesis have yet to be carried out. However, several studies already have implicated $GABA_A$ receptors and GABAergic transmission in the control of alcohol seeking and drinking (Anstee et al. 2013; Hodge et al. 1995; Hyytia and Koob 1995; June et al. 1998; Nie et al. 2011; Rewal et al. 2009). Thus, it is very likely that the effects of chronic exposure on synaptic plasticity in the NAc play a significant part in dependence and escalated drinking following such exposure.

Ultimately, each of the three striatal subregions—DMS, DLS, and NAc—contribute to the neural and behavioral changes brought about by chronic ethanol exposure. A simple model suggests that early in our experiences with alcohol, brain regions that are sensitive to the proximal relationship between actions and reward, such as the DMS and NAc, may exert strong control over alcohol seeking and drinking. With continued ethanol exposure, both internal and environmental stimuli may begin to exert greater control over alcohol seeking and drinking by strengthening brain activity in the sensorimotor and limbic circuits that are responsive to complex stimuli and predictive cues, respectively. Furthermore, relapse induced by exposure to ethanol-associated cues appears to involve the NAc and the rest of the limbic circuit and their functions in Pavlovian-instrumental transfer (Belin et al. 2009; Corbit and Janak 2007). The limbic circuit also is strongly engaged during withdrawal and abstinence from alcohol use and thus may contribute to relapse driven by the negative consequences of such abstinence (Koob 2013). The internal states and/or external stimuli that engage the sensorimotor circuitry may not only contribute to relapse, but likely also contribute to excessive ethanol intake once relapse has occurred. Indeed, once drinking has begun, the combination of the particular context and the effects of ethanol itself may drive continued intake until significant environmental or physiological

events (e.g., loss of consciousness) interfere with the habitual behavioral pattern. Future research will no doubt focus on how the different synaptic mechanisms and different brain circuits contribute to relapse and excessive drinking. Ultimately, research should strive to find ways to disrupt both processes through targeted alterations in the activity of the plasticity of the involved circuits.

Chronic Ethanol and Synaptic Plasticity in the BNST

Given the important role that the BNST plays in regulating negative affective states and negative reinforcement, several studies have examined the ability of chronic ethanol exposure to alter synaptic plasticity in this brain region. In the initial study examining chronic alcohol exposure on BNST function, and more specifically NMDAR function, Kash and colleagues (2009) found that 4 days of chronic-intermittent, but not continuous, exposure to ethanol vapor led to a functional upregulation of NR2B-containing NMDARs. The investigators also explored the temporal summation of NMDARs in response to repeated stimulation across a range of frequencies. Changes in this summation had been suggested to be an index of metaplasticity and to reflect the potential of a circuit to induce plasticity. The study found that this summation increased across all frequencies tested (Kash et al. 2009). Based on this increase in NR2B expression, the investigators hypothesized that the acute actions of ethanol on the NMDAR response (i.e., inhibition of NMDAR-mediated currents) in the BNST would be enhanced with chronic exposure. However, ethanol inhibition of NMDAR-mediated responses actually decreased after chronic exposure. This suggests that although subunit configuration may affect regulation of alcohol responsivity, other factors also are involved, consistent with the findings by other researchers (Jin and Woodward 2006; Woodward 2000; Xu and Woodward 2006; Xu et al. 2008).

Other investigators followed up on this study by examining how chronic-intermittent ethanol-vapor exposure could specifically alter plasticity. Wills and colleagues (2012) found that two cycles of chronic-intermittent ethanol exposure led to increased LTP, consistent with the model proposed in previous studies. Moreover, NR2B-containing NMDARs seemed to be upregulated at extrasynaptic sites that seemed to be coupled to LTP. These findings were in contrast to observations in the hippocampus, suggesting that novel protein signaling complexes may be associated with NMDARs in the BNST compared with other regions. Interestingly, Conrad and Winder (2011) found that adolescent ethanol exposure, when combined with exposure to stress, led to alterations in both anxiety-like behavior and LTP in the BNST, providing further support that a functional link exists between these two measures.

Silberman and colleagues (2013) found that chronic-intermittent ethanol also led to alterations in glutamatergic function in the BNST that depended on another receptor, corticotrophin-releasing factor receptor type 1 (CRFR1). This observation is consistent with studies demonstrating that CRFR1 activation could enhance glutamate release in slice preparations (Kash et al. 2008*b*; Nobis et al. 2011).

Francesconi and colleagues (2009*a,b*) also investigated the impact of chronic ethanol exposure on plasticity, specifically on the LTP of intrinsic excitability in the juxtacapsular nucleus of the BNST. As mentioned previously, this is an anatomically distinct region in the BNST, reflective of a unique set of inputs and outputs (for a review, see Lowery-Gionta and Kash 2014). In contrast to other investigators (Wills et al. 2012), Francesconi and colleagues (2009*a,b*) found that chronic alcohol exposure led to a dampening of LTP in this BNST region. Additional analyses demonstrated that the dampened LTP resulted from an upregulation of certain potassium currents (i.e., D-type potassium currents). Similar alterations in plasticity occurred after cocaine and heroin self-administration, suggesting that this may be a common adaption to chronic exposure to drugs of abuse. Finally, the chronic ethanol- induced LTP dampening was blocked by agonists to CRFR1, providing a link between the CRF systems and altered plasticity. One potential model that takes all of these changes into account posits that chronic alcohol exposure leads to increased levels of dopamine or norepinephrine in the BNST during a behavioral challenge. The increased levels of these monoamine neurotransmitters then can activate CRFR1 signaling, potentially via depolarization of CRF neurons. The resulting increase in glutamate release can act in concert with the upregulation of NR2B to lead to increased plasticity in the BNST, potentially resulting in enhanced anxiety-like behavior.

Other studies have investigated how chronic alcohol alters LTD in the BNST. Because norepinephrine is thought to play an important role in stress-induced relapse and anxiety, McElligott and colleagues (2010) investigated how chronic-intermittent ethanol altered alpha1A receptor-mediated LTD in the BNST. The investigators found that 4 days of chronic-intermittent exposure (i.e., the same exposure regimen that enhanced NMDAR function) led to a partial loss of this LTD. In contrast, 10 days of restraint stress resulted in a total loss of LTD. Reasoning that this might result from prior induction of the LTD in vivo, which occludes the induction of LTD in slices, the investigators evaluated the presence of GluR1-lacking AMPARs in the BNST. The analyses found significant downregulation of these receptors after stress, indicating in vivo induction of this form of LTD. In contrast, cocaine exposure, which alters mGluR5-LTD, did not have any effect on this

alpha1A receptor-mediated LTD. These findings support the idea that norepinephrine is released in the BNST during both stress and alcohol exposure, providing a mechanism by which the alpha1A receptor antagonist prazosin can reduce drinking and anxiety in people with alcohol use disorder (Simpson et al. 2009).

Several other studies also have examined how exposure to either drugs of abuse or stress can alter plasticity in the BNST. One series of studies focused on examining the impact of stress and nicotine exposure on CB1R-mediated plasticity. These analyses found that either stress or nicotine self-administration could reverse this LTD to an LTP (Jalabert et al. 2009; Massi et al. 2008; Puente et al. 2010; Reisiger et al. 2014). The mechanism underlying this switch in the polarity of plasticity is unclear at this point. One might hypothesize that because alcohol exposure and withdrawal are stressful, they would lead to similar changes in function; however, this has yet to be determined. Other researchers demonstrated that cocaine self-administration led to a novel LTP of GABA transmission mediated via neurotensin signaling (Krawczyk et al. 2013). Together with previous observations that cocaine can lead to CRF-dependent changes in glutamatergic transmission (Kash et al. 2008*b*; Nobis et al. 2011), this finding suggests that neuropeptide signaling may have an essential function in the regulation of plasticity in the BNST.

In summary, all of these findings indicate that alcohol can affect plasticity in the BNST; however, the specific effects likely are dependent on the subregion of the BNST and potentially even the neuronal subtype being targeted. Future work using viruses and reporter mice to specifically target molecules potentially involved in these processes will clarify and extend these results. Moreover, it is possible to move these studies beyond correlational analyses and determine the effect that these various forms of plasticity have on alcohol-related behaviors, using in vivo optogenetic[1] and chemical genetic methods. This will be essential in understanding how to target and treat discrete aspects of alcohol addiction.

Summary

Both acute and chronic ethanol exposure can modulate synaptic function and plasticity in the dorsal striatum and the BNST. Both of these regions seem to play important but distinct roles in alcohol-related behavioral plasticity. A challenge for the entire alcohol research field will be defining the molecular targets and mechanisms that mediate these ethanol-induced changes in function. It will also be critical to move beyond correlational studies and begin to define how these changes in circuits can directly regulate behavior. Elucidation of the pathways linking changes in brain plasticity to behavior hopefully also will point out potential new targets for the amelioration, reversal, or prevention of alcohol-induced changes in brain circuitry. The identification of such targets could open new avenues for translational research into novel or more effective treatment of alcohol use disorder.

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[1] Optogenetic approaches use light to control the activity of neurons that have been genetically modified so that they become responsive to light.

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Advances in Human Neuroconnectivity Research

Applications for Understanding Familial History Risk for Alcoholism

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Recent advances in brain imaging have allowed researchers to further study the networks connecting brain regions. Specifically, research examining the functioning of these networks in groups with a genetic predisposition for alcoholism has found atypical circuitry in the brains of such individuals. Further research with larger sample sizes and multimodal method integration are necessary to confirm these intriguing findings.

Key words: Alcoholism; genetic vulnerability; genetic risk factors; brain; brain function; brain imaging; neuroimaging; neuron; neural network; neuroconnectivity; neurobiology; functional magnetic resonance imaging; psychophysiological interactions; neuroscience

Advances in human neuroimaging have expanded our ability to understand the functioning of the brain, with particular recent advances fostering our analytic capacity to examine networks between the brain's nerve cells (i.e., neurons) and neuroconnectivity (i.e., neural networks). Relevant to the field of alcoholism, several researchers recently have applied these strategies to groups at genetic risk for alcoholism, in hopes of identifying neurobiological, and specifically neuroconnectivity, phenotypes underlying this risk. This article provides an overview of the methods used to study connectivity and highlights research detailing the application of these methods to studying populations at risk for alcoholism.

Neuroconnectivity Methods

Task-Based Connectivity

With the aim of understanding network functioning in the brain, one analytic strategy has been to examine the correlations between activation in regionally disparate brain regions during functional magnetic resonance imaging (fMRI).[1] To do this, investigators typically have correlated average signal change in two or more regions of interest (ROIs) during a task, with the assumption that higher correlations reflect greater connectivity between regions (i.e., they are simultaneously showing significant changes in neural activation). Although this approach is confounded by variations in the underlying baseline intrinsic connectivity of the brain, it has nonetheless been used to demonstrate evidence of altered neuroconnectivity patterns in specific populations, as detailed below.

Psychophysiological interactions (PPI) analysis is another functional connectivity method used to analyze the coupling of neuronal activity between distinct brain regions while an individual is engaged in a task. This is different than other functional connectivity methods (e.g., resting-state functional connectivity) in that it allows one to assess the impact of task condition (or context) on the functional connectivity of two distinct brain regions. Friston and colleagues (1997) first described PPI as the statistical processing (i.e., regression) of neuronal activity in one brain region (the target region) onto the neuronal activity in a second (seed) region, with the slope of this regression being indicative of the relationship between the activity in these two regions. Comparing the slope of this regression during two distinct task conditions is the crux of PPI analysis. With PPI, seed regions can either be defined by functional subsets of data (i.e., masks) created in group-level analysis by selecting the volume elements (i.e., voxels)—three-dimensional elements that make up an image—that are most active during the task condition, or by a priori selection of a particular anatomical brain ROI (O'Reilly et al. 2012). The neural activity over time from this seed region is then multiplied by the regressor representing task-related activity and entered into the individual subject model to identify brain regions or voxels whose activity is synchronous with activity in the seed region. Recently, a more generalized form of PPI analysis allowed for the use of more than two task conditions in the same PPI model (McLaren et al. 2012). This is especially pertinent when analyzing tasks that have two or more distinct experimental conditions, as well as a control (baseline) condition in which no stimulus is occurring, typical of many fMRI tasks currently employed. This generalized form of PPI allows for better within-subject model fit and prevents

[1] fMRI measures brain activity by detecting changes in blood oxygenation and flow in different areas of the brain.

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having to collapse data across multiple conditions. Furthermore, PPI also can be used to compare functional connectivity between groups using group status, instead of context, as the regressor (O'Reilly et al. 2012) and could be useful for comparing groups with and without family history of alcoholism.

Connectivity Without Task Engagement

The functional connectivity of the brain also has been measured using resting-state functional connectivity (RSFC) during fMRI. With this technique, functional connectivity is measured by correlating blood-oxygen-level–dependent (BOLD) signal, an indirect measure of neuronal activity, across the brain in an individual who is resting. Regions of the brain are thought to be functionally connected if they share a temporally correlated neurophysiologic response. Spontaneous brain fluctuations persist across a variety of states, such as sleep or anesthesia, as well as in animal species (Kannurpatti et al. 2008; Kojima et al. 2009), suggesting that spontaneous BOLD correlations are an intrinsic property of brain activity (Fox and Raichle 2007). RSFC has led to the identification of numerous sets of functionally connected brain regions, termed networks, including the default mode, fronto-parietal, dorsal attention, and ventral attention networks (Fox and Raichle 2007). These networks have been identified with a myriad of techniques, the most common of which, seed-based correlation analysis (SCA) and independent components analysis (ICA), are described below. Furthermore, analysis of complex networks using mathematical approaches (e.g., graph theory) provides insight into local and global properties of specific and whole-brain network organization (Rubinov and Sporns 2010). Such analysis can be useful for identifying differences between populations, such as those at risk for alcoholism and healthy control subjects.

SCA and ICA

SCA requires a priori selection of a voxel, cluster, or anatomical region, usually based on previous fMRI literature delineating relevant regions of activation or through anatomical delineation. From this a priori selection, time series data are extracted and used as regressors in a linear correlation (or general linear model) analysis from which whole-brain, voxel-wise functional connectivity maps are derived that co-vary with the seed region (for more details see Cole et al. 2010). This approach shows networks of regions that are most strongly functionally connected with the seed voxel or ROI. Conversely, ICA is a data-driven method of analysis that uses whole-brain data to obtain spatially independent and additive components, while assuming statistical independence of non-Gaussian source signals. Networks identified with ICA are compatible with networks found using seed-based methods and typically include less artifactual effects from noise. Additionally, this method can be effective because it eliminates some inherent bias in selecting seed regions (Cole et al. 2010).

Complex network analysis elucidates properties of neural networks beyond simple local correlations established through SCA and ICA. Complex network analysis originated from graph theory but is distinct because it deals with biological networks that are large and complex, like the brain. Nodes and links make up a complex network, which is neither random nor ordered. Nodes typically represent brain regions, whereas links can be represented by anatomical or functional connections. Nodes typically span the entirety of the cortex and do not overlap, whereas links can be unidirectional or bidirectional, or binary, or weighted and represent size, density, or coherence and magnitudes of correlations, or causal interactions in anatomical and functional networks, respectively. The relationships between nodes and edges, in turn, define the network's topology, which is amenable to descriptive analyses that explore local and global aspects of a network's organization (Sporns 2014). Node degree, clustering, and modularity are commonly applied measures.

Structural Connectivity

Neuroanatomic connectivity often is characterized with diffusion tensor imaging (DTI), which provides an indirect measure of white matter[2] integrity, including myelination and axonal coherence[3] (Hagmann et al. 2006). DTI assesses diffusion of water molecules in brain tissue. In white matter, water diffusion is restricted and preferentially diffuses along axonal bundles that make up white matter tracts. This restricted diffusion is called anisotropic. Conversely, diffusion of water molecules is isotropic, or less directionally restricted, in other tissues, such as gray matter, indicating more random diffusion. By measuring fractional anisotropy (FA) in the brain, which reflects the degree to which water diffusion is constrained, researchers can draw inferences regarding the underlying white matter microstructure. Because of limitations of DTI in accurately characterizing diffusion in regions with crossing fibers (e.g., regions of prefrontal white matter), researchers must cautiously interpret findings.

Applications of Neuroconnectivity Analyses to Studies of Risk for Alcoholism

Family History of Alcoholism and Connectivity

One major risk factor for developing an alcohol use disorder (AUD) is having a family history of alcoholism (Lieb et al. 2002; Schuckit 1985). Neuroimaging research has identified various structural and functional brain differences

[2] White matter consists of axons surrounded by a protective fatty substance (i.e., myelin) that carry information between brain cells, or neurons, which make up the brain's gray matter.

[3] Axons are the nerve fibers that carry information between neurons.

between youth with familial alcoholism and their peers using volumetric analyses, DTI, and task-based fMRI, which may suggest that there are neural markers of risk, even in the absence of heavy alcohol use (Cservenka and Nagel 2012; Cservenka et al. 2012; Herting et al. 2010; Mackiewicz Seghete et al. 2013; Schweinsburg et al. 2004; Silveri et al. 2011; Spadoni et al. 2013) and in samples with minimal abuse and dependence diagnoses (Hill et al. 2001, 2007, 2011). The available neuroconnectivity tools have been critical for identifying atypical functional connections in at-risk youth, as described below. This avenue of MRI research holds promise for characterizing brain network coherence in studies of familial and genetic risk for alcoholism and is valuable for the examination of connectivity characteristics that could predict future alcohol abuse. Assessing neuroconnectivity in those at risk for alcoholism who have not yet consumed alcohol heavily allows for the distinction between phenotypes related to risk for developing alcoholism and those that could be present as a result of alcohol-induced alterations in brain networks. This advantage may allow future prevention strategies to target their efforts toward risk phenotypes that increase vulnerability for alcoholism, prior to initiation of heavy use.

Task-Based Connectivity

Task-based functional connectivity has been used in two studies of youth with family history of alcoholism to examine connectivity during working-memory tasks. In a substance-naïve sample of 12- to 14-year-olds, Wetherill and colleagues (2012) examined functional connectivity in working-memory–relevant brain regions, including the bilateral dorsolateral prefrontal cortex (DLPFC) and the posterior parietal cortex (PPC). The BOLD time series were correlated among these seed regions during participant performance of a 6-dot version of the visual working-memory (VWM) task, in which youth had to identify whether dots were the same or different colors after a delay. All fronto-parietal connections examined exhibited weaker synchrony in youth with a family history of alcoholism (FHP) compared with their peers, despite comparable task performance between the groups. Additionally, within the FHP group, there was a significant correlation between number of missed responses and functional connectivity between the PPC and DLPFC. These findings suggest that even in the absence of alcohol or substance use, youth with familial alcoholism already exhibit similar deficits in functional connections in important executive functioning pathways as those seen in alcoholics (Schulte et al. 2012).

Some hypotheses regarding familial risk for alcoholism propose that youth with a family history of AUD may be at greater risk for alcohol abuse as a result of a developmental delay (Corral et al. 2003; Hill et al. 2001). This hypothesis was tested in a functional connectivity study that examined spatial working-memory task connectivity between predefined ROIs in FHP subjects and family history–negative (FHN) youth and compared these functional connectivity patterns to those of an older group of adolescents (Spadoni et al. 2013). Using structural equation modeling, the results showed that the FHP groups differed in connectivity in the right superior parietal lobule to left middle frontal gyrus pathway and that removal of this pathway from the model resulted in a much poorer fit for the FHP group than the FHN youth. These findings suggested that FHP youth differed from their peers in working-memory–related connectivity and that the overall fit of the model for the functional connections among working-memory–related brain regions more closely resembled older adolescents in the FHN sample. These two studies indicate that neural markers for alcoholism may be present during early adolescence when alcohol or substance use has not been initiated and that these patterns may represent a developmental delay in brain network maturity. It will be interesting for future studies to conduct graph theory analyses to examine specific metrics that may differentiate FHP and FHN adolescents in fronto-parietal executive functioning systems.

Alcoholics exhibit abnormalities in reward-related structures and atypical reward processing (Makris et al. 2008; Wrase et al. 2007), suggesting that incentive motivational systems may, in part, relate to the risk for alcohol abuse. A study of young adults used the monetary incentive delay (MID) task[2] to examine functional connectivity of the ventral striatum during incentive versus neutral trials in the MID task (Weiland et al. 2013). FHP young adults showed opposite patterns of connectivity from their peers, such that they exhibited positive functional connectivity between the ventral striatum and sensorimotor cortex, as well as default mode network regions, whereas FHN youth displayed negative functional connectivity between these regions. Additionally, positive functional connectivity between the ventral striatum and supplementary sensorimotor area (SSMA) in FHP youth was positively related to self-reported sensation seeking. A mediation analyses showed that the connectivity between the nucleus accumbens (NAcc) and SSMA mediated the significant association between sensation seeking and alcohol use in the FHP group. It is possible that increased connectivity between reward-related regions and regions involved in motor control could be maladaptive in at-risk youth. The authors proposed that this increased connectivity may represent enhanced and atypical connections between regions involved in reward salience and those important for motor preparation and action. This, in turn, could potentiate actions that involve reward-related behaviors, such as alcohol use.

Fronto-cerebellar abnormalities, including atypical connectivity between the frontal lobes and cerebellum, consistently have been reported in alcoholics (Chanraud et al. 2010; Desmond et al. 2003; Rogers et al. 2012; Sullivan et al. 2003). Because these studies often do not account for preexisting risk factors in adults with AUD, such as family history risk, Herting and colleagues (2011) examined

[4] In the monetary incentive delay task, participants respond within a time window and are potentially rewarded for the response depending on their reaction time.

fronto-cerebellar integrity in FHP youth who had no experience with alcohol to examine whether preexisting atypical connectivity of these regions may be a premorbid neural risk feature. Seed-based connectivity of these regions was examined during a variety of fMRI tasks performed by participants in the scanner, that were later averaged. The results from this study suggested weaker fronto-cerebellar connectivity in FHP youth compared with their peers, indicating that previous findings reported in alcoholics may in part be attributed to preexisting risk for alcohol abuse. Additional work is necessary to examine how the integrity of these systems relates to behavioral correlates. This will further increase understanding of the specific deficits that may be associated with weaker integrity of these functional connections, which are likely associated with executive functioning, as such functions have been reported to be mediated by fronto-cerebellar systems (Diamond 2000). Importantly, some of the networks that show FHP-associated alterations in functional connectivity include brain regions where volumetric differences have been identified in FHP individuals, such as the cerebellum (Hill et al. 2007, 2011). This suggests that the underlying basis for altered BOLD synchrony between these regions may be related to premorbid anatomical differences in these structures. Additional work using multimodal integration of structure and functional connectivity methods is needed to better understand these relationships.

Resting-State Functional Connectivity

Although more research on familial risk for alcoholism and brain connectivity has focused on functional connections present across task-related BOLD response, recent investigations have examined the intrinsic functional connectivity of brain regions in FHP youth (see figure), specifically using seed-based resting-state connectivity methods. Brain regions and networks that play important roles in reward and emotional processing (e.g., the NAcc and amygdala) often have been the focus of alcoholism research, as task-based neuroimaging studies suggest aberrant brain activity in these areas (Marinkovic et al. 2009; Wrase et al. 2007). Using anatomically defined ROIs of the NAcc on a subject-specific basis, Cservenka and colleagues (2014*a*) found significant differences in the synchrony of both left and right NAcc with other regions of the brain in FHP youth compared with their peers. Specifically, differences were most pronounced in connectivity of the ventral striatum with regions of the frontal lobe. FHP youth had less negative connectivity (or less segregation) between the NAcc and cognitive control regions of the frontal cortex, including bilateral inferior frontal gyri, than their peers. The authors suggested that because reward and executive functioning networks are not as distinctly segregated in FHP youth, this may lead to miscommunication between these regions. Furthermore, this study found that FHP youth had disrupted integration between the NAcc and orbitofrontal cortex (OFC), whereas these regions showed positive connectivity in FHN youth. The authors suggested that reward-related brain areas may be more weakly integrated in FHP youth, which may result in a dissociation between reward response in the brain (mediated by NAcc) and determining the value of rewards (mediated by OFC). Again, it is important to note that alterations in resting state synchrony between the NAcc and OFC may be related to underlying volumetric differences in these regions in FHP individuals. Disruptions in OFC laterality have been previously reported in at-risk youth/young adults (Hill et al. 2009). Associations between functional connectivity and relationships with brain structure require further study.

Recently, another study used RSFC to examine intrinsic connectivity of the amygdala in FHP adolescents and found relationships between behavior in an emotion-cognition task and functional connectivity between the left amygdala and left superior frontal gyrus (SFG) (Cservenka et al. 2014*b*). Weaker connectivity between amygdala and left SFG was associated with poorer impulse control in the context of emotional stimuli in the FHP group. The authors believe that segregation of cognitive and emotional circuitry in at-risk youth may be a marker of weaker cognitive control in FHP adolescents when they are in emotionally laden situations. Because FHN youth displayed mostly positive synchrony between these regions, which was unrelated to task performance, these findings could indicate that once connectivity is established in these regions, it may no longer aberrantly affect behavior. Given discrepancies between children and adults reported in the typical patterns of positive and negative functional connectivity between the amygdala and the frontal lobe (Qin et al. 2012; Roy et al. 2009), more work is needed to determine how the integrity of fronto-limbic circuitry is related to risk for alcoholism.

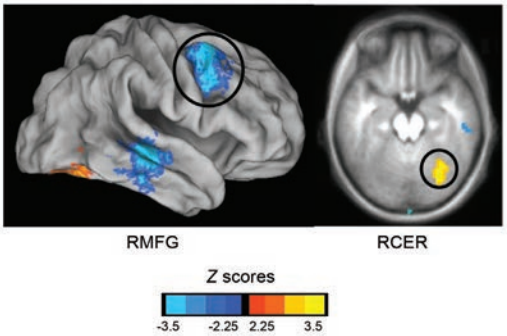

Figure FHP youth have significant differences in right amygdalar resting state functional connectivity patterns compared with FHN youth in frontal and cerebellar regions. This indicates atypical connectivity with executive functioning brain regions in at-risk adolescents compared with controls. RMFG = right middle frontal gyrus; RCER = right cerebellum.

SOURCE: Cservenka et al. 2014*b*.

Another finding from this study was an opposite pattern of functional connectivity between the amygdala and cerebellum in FHP youth compared with the pattern observed in their FHN peers (both greater and reduced connectivity, depending on the side of the brain). These results are interesting given previously reported weaker fronto-cerebellar connectivity in FHP youth (Herting et al. 2011), when BOLD signal was averaged across a variety of fMRI tasks. Not only may fronto-cerebellar connectivity be altered in FHP youth prior to heavy alcohol use, but connectivity of these regions with affect-related areas at rest also may be atypical. Interestingly, both reduced contralateral fronto-cerebellar and amygdalar-cerebellar connectivity was found across both studies (Cservenka et al. 2014*b*; Herting et al. 2011), which supports weaker interhemispheric connectivity between frontal and limbic brain regions with the cerebellum in FHP youth compared with their peers. These findings suggest that both top-down and bottom-up connections with the cerebellum show reduced synchrony across hemispheres in at-risk individuals, a phenotype that merits further exploration, especially given other reports of smaller amygdalar volumes in FHP youth (Hill et al. 2001, 2013*b*).

DTI

A number of DTI studies have identified white-matter pathways that are altered in high-risk youth and adults (Acheson et al. 2014; Herting et al. 2010; Hill et al. 2013*a*), suggesting that differences in functional connectivity may be related to atypical structural integrity of white matter in FHP individuals. The first study to do so examined white-matter integrity in alcohol-naïve FHP adolescents compared with age- and gender-matched FHN youth and found reduced FA in the superior and inferior longitudinal fasciculi, as well as the anterior superior corona radiata in FHP youth (Herting et al. 2010). Further, reduced FA mediated the relationship between familial alcoholism and reaction times on a delay-discounting task. Because many of these pathways are implicated in connections between brain regions involved in higher-order executive functioning (Seghete et al. 2013; Treit et al. 2013), the findings may reflect either a developmental delay in maturation of white matter, or more lasting deficits in white matter integrity in FHP individuals. Because executive functioning deficits have been observed in both alcoholics (Noel et al. 2007; Verdejo-Garcia et al. 2006) and offspring of alcoholics (Gierski et al. 2013; Harden and Pihl 1995; Nigg et al. 2004), it is plausible that premorbid weaknesses in top-down cognitive functioning and associated neurocircuitry could increase risk for maladaptive decisions regarding alcohol use.

Another DTI study found risk by alcohol exposure effects related to reduced FA in some of the same white-matter pathways previously reported to be altered in FHP youth, including superior and inferior longitudinal fasciculi (Hill et al. 2013*a*). Because this study was conducted in adults, it is possible that developmental timing is a key factor in determining whether risk effects alone are observed and how alcohol exposure may further compromise these vulnerable pathways.

Additional support for lower FA in a variety of white-matter tracts in frontal and parietal regions was recently reported in a large sample of 80 FHP youth, who also had lower FA, compared with their peers in anterior, superior, and posterior corona radiata (Acheson et al. 2014), with the first two pathways exhibiting similar reductions in FA to previously reported findings (Herting et al. 2010). In some cases, studies that have found reduced fronto-parietal functional connectivity in FHP youth have not found reductions in white matter integrity in fronto-parietal pathways (Wetherill et al. 2012). The dissociation between functional and structural connectivity was interpreted as delays in synaptic transmission, rather than compromised myelination of white-matter pathways (Wetherill et al. 2012). However, as a result of the small sample sizes, results need to be replicated.

Conclusions and Future Directions

As shown, several studies have used neuroconnectivity methods to identify atypical circuitry in the brains of those at familial risk for alcoholism, albeit generally with small sample sizes, which is a limitation of the available research. Overall, these studies have demonstrated abnormalities in connectivity between frontal regions with parietal, ventral striatal, cerebellar, and limbic regions of the brain in these populations, suggesting that these methods may be particularly useful in uncovering neurobiological risk phenotypes. Larger sample sizes and multi-modal method integration are critical to confirm these intriguing findings. Although studies of family history risk for alcoholism have reported atypical functional connectivity using seed-based resting-state and task-based connectivity approaches, none have used graph theory to examine network characteristics of alcoholism-related risk, an analytic strategy which may prove particularly useful for increasing our understanding of the interactions between these networks. Given recent work documenting the amenability of brain functioning to change in response to treatment (Feldstein Ewing et al. 2011), identification of neuroconnectivity treatment targets may substantially increase our capacity to intervene with at-risk populations in a neurobiologically targeted manner.

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Advances in Electrophysiological Research

Chella Kamarajan, Ph.D., and Bernice Porjesz, Ph.D.

Electrophysiological measures of brain function are effective tools to understand neurocognitive phenomena and sensitive indicators of pathophysiological processes associated with various clinical conditions, including alcoholism. Individuals with alcohol use disorder (AUD) and their high-risk offspring have consistently shown dysfunction in several electrophysiological measures in resting state (i.e., electroencephalogram) and during cognitive tasks (i.e., event-related potentials and event-related oscillations). Researchers have recently developed sophisticated signal-processing techniques to characterize different aspects of brain dynamics, which can aid in identifying the neural mechanisms underlying alcoholism and other related complex disorders. These quantitative measures of brain function also have been successfully used as endophenotypes to identify and help understand genes associated with AUD and related disorders. Translational research also is examining how brain electrophysiological measures potentially can be applied to diagnosis, prevention, and treatment.

Key words: Alcoholism; alcohol use disorder; alcohol-impaired offspring; risk factors; family risk factors; brain function; pathophysiological processes; electrophysiological research; electrophysiological measures; electroencephalogram; event-related potentials; event-related oscillations; genes; genetic factors

The discovery and recording of electrical activity (electroencephalography [EEG]) in the human brain in 1924 by the German physician Hans Berger (Collura 1993; Haas 2003) has led to numerous scientific breakthroughs and clinical applications (Borck 2005; Gloor 1994). Recording brain activity in humans using scalp electrodes provides a noninvasive, sensitive measure of ongoing brain function during resting state and during sensory and cognitive tasks (Porjesz et al. 2005). In contrast to neuroimaging methods, such as functional magnetic resonance imaging (fMRI), which have poor temporal resolution limited by the biophysics of the hemodynamic response, these electrophysiological methods have temporal resolution in the millisecond range and reflect the dynamic balance between excitation and inhibition in brain neural networks. Although fMRI methods are known to have superior spatial resolution, the data processing of neuroimaging methods (e.g., fMRI and positron emission tomography [PET]) frequently diminishes this acclaimed spatial resolution (especially during postprocessing of the data, which often involves "spatial smoothing" and/or averaging across voxels within a region of interest). In contrast, modern EEG recorded with up to 256 channels considerably improves its spatial resolution. Other comparative advantages of electrophysiological methods include (1) superior test–retest reliability of EEG within subjects and across labs, (2) relative ease of use, (3) lower cost, (4) applicability in larger studies to answer more complex and interesting questions than can not be answered with PET or fMRI, and (5) validity of EEG measures as a direct neural correlate (i.e., the EEG does not rely on an assumption about neurovascular coupling).

To date, these electrophysiological measures of brain function remain the most valuable method to study the sensory, motor, and cognitive phenomena as they unfold in the human nervous system. Scalp electrical activity results from ensembles of neurons firing in synchrony, which produce oscillatory activity. The oscillatory patterns, which have specific frequency-band characteristics, facilitate neural communication in the brain. The electrophysiological characteristics of individuals are affected by genes that control or modulate a variety of neurotransmitters and other biological factors. Electrophysiological methods have unique and far-reaching applications ranging from clinical and cognitive neuroscience to gene identification and can inform the field regarding prevention and neuropharmacological intervention in a variety of neuropsychiatric conditions, especially substance use disorders (SUDs) (Ho et al. 2010; Schuckit 2000). Further, research has firmly established the utility of electrophysiological methods in many aspects of alcoholism (for recent reviews, see Campanella et al. 2009; Pandey et al. 2012*a*; Porjesz and Rangaswamy 2007; Porjesz et al. 2005; Rangaswamy and Porjesz 2008*a,b,* 2014).

Alcoholism is a neuropsychiatric disorder with complex etiological contributions from genetic and environmental factors and their interactions (Kendler et al. 2003). Electrophysiological measures have served as effective "endophenotypes"—intermediary measures of neuropsychiatric function that are correlated with alcoholism and are involved in the pathway between genotype and alcoholism (Porjesz et al. 2005). Electrophysiological measures of brain function are highly heritable, and strong evidence suggests that some electrophysiological characteristics observed in

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alcoholics already are present in their offspring prior to exposure to alcohol or drugs, thus preceding the development of alcoholism. These electrophysiological endophenotypes may serve as valuable biomarkers for the genetic vulnerability underlying alcoholism (for reviews, see Begleiter and Porjesz 2006; Porjesz et al. 2005).

Electrophysiological activity can be recorded as continuous EEG during the resting state, reflecting ongoing mental states (Niedermeyer and Lopes da Silva 2005), or as time-locked event-related brain activity during cognitive tasks. The latter can be analyzed in the time domain as event-related brain potentials (ERPs), representing neural processing during a variety of sensory and cognitive tasks (Rugg and Coles 1996), or with newer time–frequency analyses, yielding event-related oscillations (EROs), or time- and frequency-specific oscillatory patterns during neurocognitive tasks (Basar 1999*a,b*). This article highlights recent research using EEG, ERP, and ERO methods recorded during wakeful or active states in alcoholics and in offspring of alcoholics from densely affected families (i.e., with multiple alcohol-dependent relatives), who are considered to be high-risk (HR); it summarizes the most useful and sophisticated techniques that are available for alcoholism research, and reviews advances in signal processing tools and techniques. Although acute effects of alcohol are not discussed for each of the techniques in this review (for a review, see Rangaswamy and Porjesz 2014), these studies are briefly mentioned with respect to a few of the advanced techniques that do not otherwise have any studies on alcoholics or HR subjects, in order to demonstrate some alcohol-related applications. For each method, the article also examines major findings in alcoholism and possible translational applications of these electrophysiological measures to diagnosis, prevention, treatment, and rehabilitation, including the utility of these measures as highly heritable and sensitive endophenotypic markers for gene identification, with potential for possible drug development for alcoholism.

Resting/Spontaneous EEG: Findings and Prospects

EEG records the spontaneous, continuous neural activity during various mental states and under a variety of conditions, such as eyes-closed relaxed state, eyes-open steady state, meditation, hypnosis, various stages of sleep, coma, and other normal/altered states of consciousness (Niedermeyer and Lopes da Silva 2005). EEG records a complex signal that can be decomposed into a wide range of frequencies using the Fast Fourier Transform (FFT) technique (Cooley and Tukey 1965), based on the principle that any time series can be represented as a summation of sine waves of different frequencies, each with its own phase and amplitudes (Boashash 1992). This section outlines the use of waking resting EEG power and coherence measures in alcoholics and HR offspring and discusses other novel signal processing methods using resting EEG data.

EEG Power in Alcoholism

Low-Frequency [Delta (1 to 4 Hz) and Theta (4 to 7 Hz)] Activity

Similar to phylogenic development characterized by awake delta state in reptiles and theta and alpha states in mammals (Knyazev 2012), awake EEG activity in human infancy is dominated by low-frequency delta rhythm during the first 2 years of life followed by a transition toward a gradual decrease in slow delta and theta activity as well as a gradual increase in faster alpha and beta bands almost linearly across development from childhood through adolescence to adulthood (e.g., John et al. 1980). However, increased delta activity in awake human adolescence and adulthood has been related to many neurological disorders as well as several psychiatric conditions, such as schizophrenia (Begic et al. 2000; Karson et al. 1987; Sponheim et al. 2000). In alcoholism, early EEG studies reported that abstinent alcoholics showed increased delta power (Begleiter and Platz 1972; Kaplan et al. 1985; Volavka et al. 1985). In contrast, studies have found decreased slow-wave activity in alcoholic patients in the delta band (Saletu-Zyhlarz et al. 2004) as well as in both delta and theta bands (Coutin-Churchman and Moreno 2008; Coutin-Churchman et al. 2003, 2006). Additional research among people with SUDs has reported similar findings of decreased slow-wave activity in the delta band (Alper et al. 1998), as well as in both delta and theta bands (Prichep et al. 1996). In a study of binge drinkers, Courtney and Polich (2010) reported that high–binge drinkers exhibited more spectral power in the delta (0 to 4 Hz) and fast-beta (20 to 35 Hz) bands than non– and low–binge drinkers. Taken together, findings on delta power in alcoholism seem to be inconclusive.

Human resting theta rhythm has its maximum power in the posterior scalp region; the normal adult waking EEG record contains a relatively lower amount of theta power compared with other fast frequencies (cf. Porjesz et al. 2005). Studies have reported that alcoholic subjects manifest increased resting theta power (Fein and Allen 2005; Propping et al. 1981, 1992; Rangaswamy et al. 2003), although some studies by Coutin-Churchman and colleagues (2003, 2006) have reported decreased theta activity in alcoholics. It is also interesting to note that HR offspring of alcoholics from densely affected families do not manifest the abnormal theta power seen in alcoholics, in contrast to several other EEG measures. Hence these theta abnormalities in alcoholics are likely the result of chronic alcohol intake on brain function (Rangaswamy and Porjesz 2014). As reviewed below ("Electrophysiological Measures as Endophenotypes for Alcoholism"), genetic research has found linkage and association of a cholinergic muscarinic neurotransmitter receptor gene (*CHRM2*) with two theta oscillation measures: (1) theta ERO during the processing

of target stimuli during an oddball task 1 and (2) resting eyes-closed EEG high-theta (6 to 7 Hz) interhemispheric coherence (Jones et al. 2004, 2006*a*; Porjesz and Rangaswamy 2007; Rangaswamy and Porjesz 2008*b*).

Alpha Band (8 to 12 Hz)

The alpha rhythm is predominant when an individual is awake and relaxed, and has its maximum power in the eyes-closed condition over the occipital regions. Human alpha oscillations (during resting state as well as during cognitive processing) are related to higher cognitive function and brain maturation. Alpha activity in children starts only after 3 years of age, almost parallel to the development of speech, and the posterior dominant alpha rhythm continues to develop until the age of 16 (cf. Basar 2012). Many early EEG studies showed that alcoholics manifest less prevalent and lower alpha power compared with control subjects (for reviews, see Begleiter and Platz 1972; Propping et al. 1981). However, some studies failed to replicate this finding of low resting alpha power in alcoholics (Enoch et al. 1999; Fein and Allen 2005; Pollock et al. 1992). Researchers found that a decrease in slow alpha activity in alcoholics is more pronounced in relapsers than in those who maintain abstinence (Saletu-Zyhlarz et al. 2004). Further, gender- as well as ethnicity-related alpha findings have been reported in offspring of alcoholics (Ehlers and Phillips 2003; Ehlers and Schuckit 1991; Ehlers et al. 1996; Finn and Justus 1999). Manifestations of the low-voltage alpha (LVA) variants may be influenced by ethnicity and gender, whereas the findings on alpha power are equivocal. The association of LVA variants in females to a catechol-o-methyltransferase (COMT) gene and to anxiety and alcoholism is discussed in a later section (see "Electrophysiological Measures as Endophenotypes for Alcoholism").

Beta Band (12 to 28 Hz)

Beta-frequency rhythms in resting EEG are prevalent in the awake and alert state. Several studies have reported increased beta power in the resting EEG of alcoholics (Bauer 2001; Costa and Bauer 1997; Fein and Allen 2005; Propping et al. 1981; Rangaswamy et al. 2002; Winterer et al. 1998). Increased beta activity often is taken as a sign of increased neural excitability (hyperexcitability or central nervous system [CNS] disinhibition); it is apparent in alcoholics (Porjesz et al. 2005), where it has been shown to be a predictor of relapse (Bauer 2001; Saletu-Zyhlarz et al. 2004), and has been reported in HR relatives of alcoholics, including both male and female offspring (Finn and Justus 1999; Gabrielli et al. 1982; Pollock et al. 1995; Rangaswamy et al. 2004*b*), although it is more robust in males (Finn and Justus 1999; Gabrielli et al. 1982; Pollock et al. 1995; Rangaswamy et al. 2002, 2004*b*). This suggests that the neural hyperexcitability observed in alcoholics may antecede the development of alcoholism (Porjesz et al. 2005). The association of beta and neural hyperexcitability to a gamma-aminobutyric acid type A ($GABA_A$) receptor gene (*GABRA2*) is discussed below (see "Electrophysiological Measures as Endophenotypes for Alcoholism").

[1] In an oddball task, participants are presented with a continuous stream of auditory or visual stimuli and are required to only respond to the presence of a designated "target" stimulus—a relatively infrequent stimulus (i.e., the oddball stimulus), while ignoring and not responding to all other stimuli (i.e., non-targets).

EEG Methods and Advances

Dipole Source Modeling for EEG Data (FFT Dipole Approximation)

Following the rapid growth of quantitative EEG (qEEG) and digital signal processing in the late 1960s, several methods to track neural generators of EEG and ERPs were introduced (see the section "Dipole Source Modeling for ERP Data"). Dipole source modeling is one of the early techniques attempting to solve the inverse problem of deriving the source configuration from recorded scalp potentials, by using mathematical simulations and modeling to understand the spatiotemporal complexity of both ongoing and evoked electrical scalp activity (Lehmann and Michel 1989; Scherg 1990; Scherg and Berg 1996; Scherg and Picton 1991) (for a detailed account on source localization methods, see Pizzagalli 2007). The cerebral sources of EEG/MEG data are estimated using mathematical modeling approaches. Specifically, for EEG data, researchers introduced a prominent method known as the FFT dipole approximation model (FFT-DA) (Lehmann and Michel 1989; Michel et al. 1992). The FFT-DA method enabled the computation of intracerebral, three-dimensional location of single dipole sources by modeling multichannel EEG data in the frequency domain using a potential distribution map containing polarity and phase information. This approach has been used predominantly to compute intracerebral sources of various EEG frequency bands in clinical conditions, such as schizophrenia (Dierks et al. 1995), depression (Dierks et al. 1993), Alzheimer's disease (Huang et al. 2000), and epilepsy (Ebersole 1991; Verhellen and Boon 2007). Although there are no studies on EEG dipole modeling in alcoholism, it may be worth revisiting this method, as dipole modeling in EP/ERP data has been successfully applied to alcoholics (Hegerl et al. 1995). Dipole modeling algorithms have been often criticized as making unrealistic assumptions about the number of likely generators and their size or orientation (Bauer 2001). Further, when the assumption of a single oscillating dipole generator is unwarranted or unlikely, resulting source identification may be less reliable (Pizzagalli 2007).

Resting EEG Coherence

Coherence is a measure of "coupling" or functional association between two brain regions (Nunez 1981, 1995). Coherence between distant brain regions is related to higher-order cognitive function, is specific to mammalian and human

brains, and does not occur in the neural networks of invertebrates and other lower animals (Basar and Guntekin 2009; Bullock and Basar 1988). Measuring coherence with the objective of discovering groups of neurons that act together in a coherent fashion (i.e., Hebbian cell assemblies) (Hebb 1949), has a long history (Horwitz 2003). EEG coherence reflects the dynamic functional interrelation between spatially separated electrode sites (Horwitz 2003). Coherence is computed as a normalized coefficient of cross-spectral power between two signals, and it estimates the consistency of phase, weighted by amplitude, between any pair of signals for each frequency (cf. Srinivasan et al. 2007). As a noninvasive method at the macroscopic level, EEG was the first method to examine the functional connectivity between different cortical regions, by correlating different features of the spatiotemporal waveforms associated with measured electrical activity using several techniques (Adey et al. 1961; Barlow and Brazier 1954; Gevins et al. 1985; Livanov 1977; Pfurtscheller and Andrew 1999). For instance, Gevins and colleagues (1985) measured dynamically changing cross-correlation of the time series between a pair of electrodes; Pfurtscheller and Andrew (1999) computed the correlation in the frequency domain between EEG signals at different scalp sites. Chorlian and colleagues (2009) reported frequency-specific topographical patterns in bipolar EEG coherence (which is devoid of volume conduction effects), and found an interesting similarity of these patterns with those obtained by resting state networks identified by fMRI studies.

EEG coherence has been used to examine cognitive and emotional processes (Kislova and Rusalova 2009; Marosi et al. 1999; Martin-Loeches et al. 2001; Thatcher et al. 2005), cognitive impairment (Babiloni et al. 2010; Gasser et al. 2003; Marosi et al. 1997), and various clinical conditions (Barry et al. 2005; De Vico Fallani et al. 2010; John 2009; Kumar et al. 2009; Shaw et al. 1983). It also has been used to index brain maturation (Barry et al. 2004; Gasser et al. 2003; Hanlon et al. 2005; Thatcher 1992, 1998; Thatcher et al. 1987, 2008). Gender differences in coherence also have been observed (Hanlon et al. 1999; Koles et al. 2010).

Increased EEG coherence in slower frequencies (e.g., delta band) and decreased coherence in higher frequencies (e.g., high alpha and beta bands) have been reported in alcoholics (Kaplan et al. 1985; Tcheslavski and Gonen 2012). Michael and colleagues (1993) found increased delta coherence (F3 to F4); increased fast-beta coherence (F3 to F4 and C3 to C4); and also reported an increase in theta, alpha, and slow-beta coherence at a central electrode pair (C3 to C4). They also found that alcohol-naïve first-degree relatives of alcoholics had shown significantly higher alpha and beta coherence than alcoholics (frontal and parietal regions) and healthy control subjects (frontal and centroparietal regions). However, there are no follow-up studies to confirm these findings in HR subjects. Winterer and colleagues (2003) reported that bilateral, intrahemispheric, posterior coherences were significantly increased in the alpha and beta bands in both long-term abstinent and nonabstinent alcohol-dependent study participants, particularly when depressiveness was included as a covariate. Abstinent alcoholics also have been reported to manifest increased resting interhemispheric high theta (6 to 7 Hz) coherence with a more posterior topography than control subjects (Porjesz and Rangaswamy 2007; Rangaswamy and Porjesz 2008b). This high-theta coherence phenotype was found to be associated with both a $GABA_A$ (*GABRA2*) and a cholinergic receptor (*CHRM2*) gene (see "Electrophysiological Measures as Endophenotypes for Alcoholism").

Graph Theoretical Methods

From a graph theoretical perspective, the brain is conceptualized as a networked system composed of regions (nodes) functionally connected with different brain regions by "paths," which are weighted by measures of statistical dependencies between their electrical activity at the nodes they connect (De Vico Fallani et al. 2012). Some properties of interest are whether there are groups of nodes more strongly connected to other nodes in their group than to nodes in other groups, whether there are paths of high connectivity between most nodes, and whether some nodes (hubs) have many paths of high connectivity with other nodes. Graph theoretical analysis offers a powerful way to understand the topological principles of brain networks in normal and clinical populations and across development (He and Evans 2010). These methods have been applied to resting state as well as task-related data (Reijneveld et al. 2007). Magnetoencephalographic (MEG) studies also have used graph theoretical methods. Although EEG and MEG signals originate from the same neurophysiological processes, magnetic fields recorded using scalp sensors are less distorted than electrical potentials by the skull and scalp. However, MEG detects only tangential, intracellular currents, whereas EEG is related to both radial and tangential extracellular currents emanating from cortical sulci and gyri (Babiloni et al. 2009; Cohen and Cuffin 1983). Using the graph theoretical methods with EEG/MEG data, researchers have analyzed brain networks in several clinical conditions, including schizophrenia (Bassett et al. 2009; Rubinov et al. 2009), epilepsy (van Dellen et al. 2009), depression (Leistedt et al. 2009), and bipolar disorder (Kim et al. 2013). In an MEG study using graph theoretical approaches, Stam and colleagues (2009) showed that patients with Alzheimer's disease had decreased connectivity of hubs in their brain networks and that highly connected neural network hubs were especially at risk in this disease. This result also was compatible with a previous fMRI-based brain network study in Alzheimer's disease (Supekar et al. 2008). In alcoholism, Sakkalis and Marias (2012) elicited statistically significant graph-theoretic indices that quantified cognitive processes in the EEG data of alcoholic subjects. Sakkalis and colleagues (2014) found that alcoholics had impaired (graph theory based) synchronization and loss of lateralization during the rehearsal process, most prominently in alpha (8 to 12 Hz) and beta (13 to 30 Hz) bands, compared with

control subjects. Further studies are under way in alcoholics and HR offspring.

Microstate Analysis

The topographic distributions of the EEG scalp potential during resting state does not change randomly or continuously over time but remains stable over periods of about 100 to 200 ms; these quasi-stable topographic distributions of the electrical field potential have been termed "microstates" (Lehmann et al. 1998) and are considered to be "atoms of thought" (Lehmann et al. 2004). It has been proposed that there is an intrinsic connection between the fast neuronal activity and slow hemodynamic fluctuations as revealed by concurrent EEG and blood oxygenation level–dependent (BOLD)-fMRI studies (Britz et al. 2010; Musso et al. 2010). Therefore, sequences of EEG microstates are assumed to be electrophysiological signatures of resting state networks of the BOLD signals (Yuan et al. 2012). Microstate analysis yields a repertoire of short-lasting functional states, termed "classes," described by topographic pattern, occurrence frequency, duration, and temporal sequence, or "syntax" (Koenig et al. 2002; Schlegel et al. 2012). These variables showed a lawful, complex evolution with advancing age from early childhood to late adulthood (Koenig et al. 2002).

Microstate analyses have been implemented in several clinical conditions, including schizophrenia (Lehmann et al. 2005; Nishida et al. 2013; Strelets et al. 2003), depression (Strik et al. 1995), attention deficits in children (Brandeis et al. 1998; Latchoumane et al. 2013), and panic disorder (Kikuchi et al. 2011; Wiedemann et al. 1998). Although no studies have been done to date, microstate analyses may be potentially useful in alcoholism as well.

ERPs: Findings and Prospects

ERPs are time-locked voltage fluctuations of the scalp-recorded neuroelectric activity in response to a sensory, motor, or cognitive event, extracted by signal processing methods such as filtering and trial averaging (Picton et al. 2000). These electrical potentials are obtained by averaging single trial EEG epochs time locked to a stimulus or event and represent large numbers of neural elements acting in synchrony during information processing, from early sensory perception to higher cognitive processing. Early components (less than 100 ms) index sensory reception, whereas the later components (more than 100 ms) index higher cognitive processing, such as selective attention, memory updating, semantic comprehension, and other cognitive activity (Duncan et al. 2009). ERP components are identified and interpreted based on their eliciting conditions, polarity (positivity or negativity), timing (latency), and scalp distribution or topography (cf. Kamarajan and Porjesz 2012). The latency (time of occurrence of an ERP phenomenon in milliseconds) reflects neural processing time, whereas the amplitude (magnitude of an ERP component in microvolts) has been related to the neural resources available to process a stimulus or event (Rugg and Coles 1996). Frequently studied ERP components include P1, N1, P2, N2, P3 (P300), N4 (N400), mismatch negativity (MMN), contingent negative variation (CNV), and bereitschaftspotential (BP) or readiness potential. These components are obtained using specific ERP tasks, such as oddball tasks, Go/No-Go tasks,[2] continuous performance task (CPT),[3] stop-signal tasks,[4] monetary gambling tasks (MGT), decision-making tasks, memory tasks, and tapping (motor) tasks. The following section reviews salient ERP findings reflecting neurocognitive (dys) function in alcoholics and HR offspring of alcoholics and discusses the application of source localization methods (e.g., dipole modeling, current source density [CSD], low-resolution brain electromagnetic tomography [LORETA]) and componential methods (e.g., principal component analysis [PCA], independent component analysis [ICA], trilinear modeling).

ERP Deficits in Alcoholism

Sensory and Perceptual Processing (Brainstem Sensory Potentials and P1/P100)

Sensory potentials are the voltage changes recorded in the brain in response to a sensory stimulus, representing the information flow along the pathway from the sense organ to the brain in response to an external stimulus, providing quantitative measures of the functional integrity of the sensory pathways (Zaher 2012). Chronic alcoholics have been reported to have prolonged latency in the auditory brainstem sensory potentials, fast time-locked potentials recorded at the scalp that represent processing along the auditory brainstem pathway (Begleiter et al. 1981; Chan et al. 1985; for a review, see Porjesz and Begleiter 1993). However, these abnormalities in early brainstem components recovered after a period of abstinence (Porjesz and Begleiter 1985) and were not found in HR individuals (Begleiter et al. 1987*a*), suggesting that they are related to the lifetime dose of alcohol consumption (Begleiter et al. 1987*a*; Nicolas et al. 1997). The P1 component of the ERP is a positive-going potential occurring around 100 ms after stimulus onset. P1 represents the basic perceptual processing of the stimulus (Heinze and Mangun 1995) and also is sensitive to various task demands

[2] In Go/No-Go Tasks, stimuli are presented in a continuous stream and participants perform a binary decision on each stimulus. One of the outcomes requires participants to make a motor response (Go), whereas the other requires participants to withhold a response (No-Go). Accuracy and reaction time are measured for each event. Go events typically occur with higher frequency than No-Go events.

[3] In a CPT, subjects are presented with a stream of letters and must respond to one of the letters (e.g., "X") only when it follows a specific letter (e.g., "O") and refrain from responding to any other combination of letters (e.g., in the case of "X" preceded by any other letter of the alphabet, or any other letter following "O", no response is made).This is called an "O-X" paradigm.

[4] In stop-signal tasks, participants have to respond as quickly as possible to a particular stimulus feature (e.g. color, shape, identity, or location). On a minority of the trials, the go stimulus is followed by an additional signal (e.g. an auditory tone or a visual cue), which instructs participants to withhold their planned response.

(Taylor 2002). Decreased P1 amplitude (Chan et al. 1986; Maurage et al. 2007; Nicolas et al. 1997), delayed latency (Cadaveira et al. 1991; Chan et al. 1986; Fein et al. 2010), and topographic changes (Miyazato and Ogura 1993) of the P1 component, particularly in visual paradigms, have been observed in chronic alcoholics.

Taken together, early sensory deficits indexed by electrophysiological measures, such as brainstem potentials, seem to be a result of the direct effects of chronic alcohol intake and recover with prolonged abstinence, whereas some later cognitive components, such as P3, do not recover (Porjesz and Begleiter 1985) and may antecede the development of alcoholism (see ERP section on P3).

Selective Attention (N1/N100)

The N1 or N100 component occurs around 100 ms after the stimulus and represents selective attentional processing; it has been shown to be modulated by the cognitive or emotional salience of the stimulus (Haider et al. 1964; Hansen and Hillyard 1980; Mangun and Hillyard 1995), and a larger N1 component is elicited for the attended and/or salient stimuli (Talsma and Woldorff 2005; Vogel and Luck 2000). Diminished N1 component has been found in both alcoholics (e.g., Cohen et al. 2002; Patterson et al. 1987) and their first-degree relatives (Steinhauer et al. 1987). Whereas the suppressed N1 component in alcoholics and HR study participants may indicate poor attentional modulation during stimulus processing, replication studies with identical methodology are required to confirm the phenomenon of N1-related deficits.

Automatic Stimulus Change Detection: MMN

Another early occurring ERP component investigated in alcoholism research is the MMN, which is a powerful measure of automatic central auditory processing (Naatanen et al. 2007). MMN is typically evoked by a physically deviant auditory stimulus and occurs between 170 and 240 ms after stimulus onset (Giard et al. 1990), reaching maximal amplitude at frontal scalp locations (Naatanen and Alho 1995). In alcoholism, MMN findings are equivocal. Although some studies reported larger MMN in alcoholics (e.g., Ahveninen et al. 2000) and in HR subjects (e.g., Zhang et al. 2001), others have failed to find any MMN-related changes in alcoholics (Fein et al. 2004*a*,*b*) and in HR individuals (Rodriguez Holguin et al. 1998; van der Stelt et al. 1997). Deficiencies in MMN may be related to deficits in central auditory processing (Naatanen 1995) and impairments in neural systems related to automatic stimulus change detector mechanisms, possibly involving frontal lobes (Alho et al. 1994). More studies are needed to ascertain and characterize the MMN related deficits in alcoholism.

Error-Related Negativity (ERN/Ne)

Error-related negativity (ERN, or Ne) is a large negative potential observed within 50 to 200 ms (and peaking around 150 ms) after an "incorrect" response in tasks that require "correct" identification of a stimulus presented (Falkenstein et al. 1991; Gehring et al. 1993, 1995; Holroyd et al. 1998). ERN is an electrophysiological index of error monitoring, or detection of the discrepancy between the desired and actually executed action, and is generated in the anterior cingulate cortex (Carter et al. 1998; Debener et al. 2005*b*). Whereas ERN is a preconscious mechanism, a later positive component, termed "error positivity" or Pe, occurring around 300 ms, is related to conscious awareness of the error (Davies et al. 2001; Overbeek et al. 2005). ERN amplitude has been reported to be lower in individuals with schizophrenia (Alain et al. 2002; Bates et al. 2002), opiate dependence (Forman et al. 2004), cocaine dependence/use (Franken et al. 2007; Hester et al. 2007), and externalizing traits such as aggression, bullying, and defiance (Hall et al. 2007), and higher in individuals with obsessive-compulsive disorder (Hajcak and Simons 2002; Johannes et al. 2001) and anxiety traits (Hajcak et al. 2003). Studies have shown that acute alcohol administration significantly reduced ERN amplitude (Bailey et al. 2014; Bartholow et al. 2012; Easdon et al. 2005; Holroyd and Yeung 2003; Ridderinkhof et al. 2002) (for a review on ERN and psychopathology, see Olvet and Hajcak 2008)]. Similarly, heavy drinkers also displayed a smaller ERN amplitude (Bartholow et al. 2012). By contrast, an ERN study (using an error paradigm) in alcoholism reported that ERN amplitudes were increased for alcohol-dependent patients compared with healthy control subjects, particularly in patients with comorbid anxiety disorders (Schellekens et al. 2010). As reviewed in the next sections (as part of N2 and P3 components of ERPs), given the findings that reduced feedback-related negativity (i.e., N2 during loss or gain) in reward paradigms was observed in alcoholics (Kamarajan et al. 2010) and those with a family history of alcoholism (Fein and Chang 2008), more studies are necessary to confirm findings from Schellekens and colleagues (2010) as well as to establish ERN changes in alcohol and other SUDs.

Attentional Orientation and Conflict Monitoring (N2/N200)

The N2 is a negative going wave observed approximately 200 to 350 ms after stimulus onset, maximally at frontocentral sites, and has been associated with several processes such as the covert orienting of attention, the detection of response conflict (conflict monitoring), response inhibition, and error detection (Jodo and Kayama 1992; Nieuwenhuis et al. 2004; Wijers et al. 1989). Alcoholics have been reported to have longer N2 latency (Cadaveira et al. 1991; Porjesz et al. 1987) and lower amplitude (Cristini et al. 2003; Realmuto et al. 1993) during an oddball task. Decreased N2 amplitude in alcoholics also has been observed during inhibition in a Go/No-Go task (Cristini et al. 2003; Pandey et al. 2012*b*) and during loss in a MGT task (Kamarajan et al. 2010). Further, Fein and Chang (2008) reported that

smaller N2 amplitudes in feedback trials were associated with a greater family history density of alcohol problems.

Target/Context Processing, Inhibitory/Cognitive Control, and Feedback Processing (P3/P300)

The most robust electrophysiological findings in alcoholism are related to the P3 component (Porjesz et al. 2005), a large positive going wave that occurs between 300 and 600 ms after the stimulus (Duncan et al. 2009; Sutton et al. 1965). P3 is not related to the physical characteristics of the stimulus but is related to its "significance" and is an index of various neurocognitive processes, including attention and working memory (Donchin 1981; Kok 2001; Polich 2007; Verleger 1988).

A large body of research has established that alcoholics consistently manifest significantly lower P3 amplitudes under a variety of task conditions and in both genders (for reviews, see Begleiter and Porjesz 1990b; Campanella et al. 2009; Porjesz and Begleiter 1993, 2003; Porjesz et al. 2005). The reduced P3 amplitude in alcoholics does not recover with prolonged abstinence (Porjesz and Begleiter 1985) and has been found to be related to the number of first-degree alcoholic relatives more than the drinking history of an alcoholic (Cohen et al. 1995; Pfefferbaum et al. 1991) or of an HR individual (Benegal et al. 1995). Furthermore, low P3 amplitude in prepubescence has been shown to predict later substance abuse, including alcohol abuse in adolescence (Berman et al. 1993; Hill et al. 1995; Iacono et al. 2002, 2003).

Another body of research shows that, similar to alcoholics, HR offspring manifest significantly lower P3 amplitude under a variety of task conditions (for reviews, see Begleiter and Porjesz 1990*a,b*; Porjesz and Begleiter 1990, 1991, 1997; Porjesz et al. 2005). Since the initial report of low P3 amplitudes in young sons of alcoholics prior to any exposure to alcohol (Begleiter et al. 1984), P3 deficits have been reported in both male and female children, adolescent, and young adult HR offspring (cf. Porjesz et al. 2005). It has been hypothesized that reduced P3 reflects underlying neural disinhibition (i.e., hyperexcitability), which in turn may be involved in the predisposition to alcoholism (Begleiter and Porjesz 1999). These findings underscore the utility of P3 as an effective endophenotype in alcoholism. (See more discussion on endophenotypes and genetic findings in a later section, "Electrophysiological Measures as Endophenotypes for Alcoholism.")

Language Processing (N4/N400)

N400 is a negative component, occurring around 400 ms (within a 300- to 650-ms window) and predominantly over the centro-parietal scalp region, in response to a semantically incongruent or inappropriate stimulus (for review, see Kutas and Van Petten 1988). N400 in ERP paradigms can be obtained either by presenting sentences with semantically deviant words or by presenting a series of words with a priming effect. A word is responded to more quickly and accurately if it is preceded by similar or related words (primed) than if it follows dissimilar or unrelated words. In normal subjects, unprimed words elicit larger N400s than primed words, whereas N400 for primed words are either small or absent (Kutas and Hillyard 1989). N400 deficits have been reported in several neuropsychiatric and cognitive disorders (Olichney 2013), especially in schizophrenia (Mohammad and DeLisi 2013). In the first study using a semantic priming paradigm in alcoholics, it was reported that alcoholics exhibited an N400 component for both primed and unprimed words, whereas the control subjects elicited N400 only for unprimed words (cf. Porjesz and Begleiter 1995). Using a sentence paradigm, reduced amplitudes in alcohol-dependent subjects (Ceballos et al. 2003, 2005; Nixon et al. 2002) were reported. In a priming study (where some of the words were antonym pairs), Roopesh and colleagues (2010) reported that although control subjects showed significant attenuation of the N400 response to the primed word compared with the unprimed word, alcoholics did not show this differentiation. Similar results of lack of attenuation to primed stimuli were found with the same paradigm in HR offspring (Roopesh et al. 2009). These findings indicate that alcoholics and HR offspring manifest inefficient neural processing, responding similarly regardless of stimulus and task requirements.

Advances in ERP Methods

Source Localization Methods

Although brain electrical activity recorded from scalp EEG has high temporal resolution on the scale of milliseconds, the spatial resolution can be limited, as cortical electrical activity is blurred over the scalp when volume conducting through the low conductivity skull (He et al. 2001; Nunez 1981; Srinivasan 1999). Several attempts have been made to improve the spatial resolution of the scalp EEG by using source localization techniques that employ computational algorithms to "de-blur" the recorded scalp potentials. (For a review on methods, see Grech et al. 2008). The most commonly used source localization methods are discussed below.

Dipole Source Modeling for the ERP Data

Dipole source analysis, as a tool to identify the generation of neuronal structures and to separate overlapping activity, also has been applied to analyze scalp-recorded ERPs. It mainly has been applied to P3(00) data (Hegerl and Frodl-Bauch 1997) to understand the sources of P300 activity (Tarkka et al. 1995) and to separate and enhance the reliability of overlapping sources of P300 subcomponents (Hegerl and Frodl-Bauch 1997). A variety of dipole source analysis methods often are performed using the software brain electrical source analysis (BESA) (Miltner et al. 1994).

Dipole modeling techniques permit estimates of underlying brain sources of scalp-recorded potentials, thus helping to interpret ERP findings with respect to those obtained from other methods (e.g., fMRI, PET or brain lesion studies) (cf. Amodio et al. 2014). Dipole source analyses have been implemented to identify sources and deficits of ERP potentials in schizophrenia (Oknina et al. 2005; Youn et al. 2003), depression (Li et al. 2011), anxiety (Li et al. 2011), obsessive-compulsive disorder (Kim et al. 2006, 2009), drug use (Mejias et al. 2005; Tuchtenhagen et al. 2000; Wan et al. 2009), and AUD (Hegerl et al. 1996*a*; Tarkka et al. 2001). In alcoholism, an increase in the intensity dependence (i.e., corresponding amplitude change based on stimulus intensity) of the tangential dipole for the N1/P2 component was observed in alcoholics, whereas a decrease was found in healthy control subjects (Hegerl et al. 1995, 1996*a*). Tarkka and colleagues (2001) performed dipole source analysis of ERPs related to automatic auditory processing (i.e., MMN) and found that processing of alerting tones was located at frontal regions in violent alcoholics, whereas the same processing was identified at medial temporal regions in nonviolent alcoholics and normal subjects. Similarly, dipole modeling has identified changes in the location of brain sources for P50, P100, and MMN components in alcoholics (Pekkonen et al. 1998).

Current Source Density (CSD)

EEG recorded with scalp electrodes represents summated activity from multiple brain sources and not just the source activity close to the electrode location. An estimate of the local radial current density or CSD for the EEG activity is normally calculated using a surface Laplacian method, an algorithm first implemented by Hjorth (1975), to improve spatial resolution and eliminate the influence of reference electrode distortions. Surface Laplacian reflects the radial projections of underlying current sources within the brain, and represents a unique, unambiguous measure of neuronal activity at scalp by providing estimates of local current flux from the brain through the skull into the scalp (Tenke and Kayser 2012). The surface Laplacian mainly acts as a spatial filter, and provides a more local representation of electrophysiological activity than the directly recorded potential (Hjorth 1975; Nunez 1981; Wang and Begleiter 1999). The CSD creates neuronal generator patterns contributing to scalp-recorded EEG in terms of local sources (positivity that represents the current flow from the brain to the scalp) and sinks (negativity that indicates the current flow from the scalp to the brain) and thus offers insights into the anatomical origins of the scalp potentials (Tenke and Kayser 2012). However, there are many methods for computing surface Laplacians of brain potentials. Local methods interpolate potentials only from the surrounding electrodes (Hjorth 1975), whereas global methods use all the electrodes by constructing a global potential function, so that the Laplacian at any point depends on the potentials at all electrodes. Interpolation can be implemented using the spherical spline method (Perrin et al. 1987). Further information on the methods and algorithms are detailed elsewhere (Nunez 1989; Srinivasan et al. 1996; Wang and Begleiter 1999).

CSD methods, using ERP data, have been successfully applied to several neuropsychiatric conditions, including alcoholism, to elucidate differences in source activations during cognitive processing (Kamarajan et al., 2014, in press). Adult alcoholics manifest low P3-related source activations during the performance of oddball tasks (Cohen et al. 2002; Hada et al. 2000; Rodriguez Holguin et al. 1999*a*) and showed changes in topographic activation patterns related to response inhibition (Kamarajan et al. 2005*a*), reward evaluation (Kamarajan et al. 2012), and language processing (Roopesh et al. 2010). Similar lower activations of P3 sources, as well as differences in CSD topographic patterns, have been reported in HR offspring of alcoholic parents (Hada et al. 2001; Ramachandran et al. 1996; Rodriguez Holguin et al. 1999*b*).

CSD studies in alcoholism also revealed region-specific activations and altered topographic features. In a visual category-matching task, Ji and colleagues (1999) reported suppressed activations at the left temporal-occipital areas in alcoholics during both matching and nonmatching conditions (around 250 ms). In a Go/No-Go task, Kamarajan and colleagues (2005*a*) found that alcoholics had lower P3 amplitudes and a more diffuse and weaker P3 source without the prefrontal sink, which was observed in the control subjects during the No-Go condition (see figure 1, panels A1 and A2). Further, Kamarajan and colleagues (2012) compared topographic patterns of ERO theta activity representing total theta power with CSD maps computed from theta amplitude data extracted within the time interval of 200 to 500 ms during the feedback of loss and gain during a single-outcome monetary gambling task, with a bet of either 10 cents or 50 cents, and found low theta power and lower CSD activations in alcoholics along with topographic differences between groups (see figure 1, panels B1 and B2).

LORETA

LORETA is a functional imaging method to localize source activations of the scalp-recorded EEG/ERP potentials by mapping the activations in three-dimensional volume elements (voxels) in the digitized Talairach atlas (Pascual-Marqui et al. 1994). This method has been further elaborated as standardized LORETA or sLORETA (Pascual-Marqui 2002) and exact LORETA or eLORETA, with reportedly improved algorithm and other tools (Pascual-Marqui 2007). The LORETA method has been widely used to understand brain activation patterns during cognitive processing in healthy study participants as well as in several clinical conditions (Pascual-Marqui et al. 2002), as shown in its Web site: http://www.uzh.ch/keyinst/loreta.htm.

Several studies have used LORETA methods to investigate cognitive dysfunction in alcoholics and HR offspring. Prabhu and colleagues (2001) reported that source

localization of visual P3 showed decreased activation in female alcoholics compared with control female social drinkers in right dorsolateral prefrontal cortex and ventromedial frontocentral regions. Chen and colleagues (2007) found significantly reduced P3-related current density activation in frontal regions (anterior cingulate, medial, and superior frontal) in alcoholic study participants while processing target stimuli in a visual oddball task. Alcoholics scored higher on impulsivity, and highly impulsive participants had the lowest activations in these areas. In a Go/No-Go task, Kamarajan and colleagues (2005*b*) found that offspring of alcoholics exhibited reduced activation in frontal, anterior cingulate, and temperoparietal regions during the P3 activity of the No-Go condition.

Using sLORETA in a Go/No-Go task, Pandey and colleagues (2012*b*) reported significantly smaller N2-related activations during the No-Go condition at bilateral anterior prefrontal regions in alcoholics compared with control subjects (see figure 2). Further, sLORETA analysis in a MGT task revealed that alcoholics, as compared with control subjects, showed significantly lower P3-related current density activations at cingulate gyrus, along with significantly reduced N2-related current density at postcentral gyrus, inferior frontal gyrus, and precentral gyrus during both loss and gain conditions (Kamarajan et al. 2010) (see figure 2). These studies demonstrate the utility of LORETA methods in revealing the activity patterns of key brain regions that are associated with neurocognitive dysfunction in alcoholics and HR offspring.

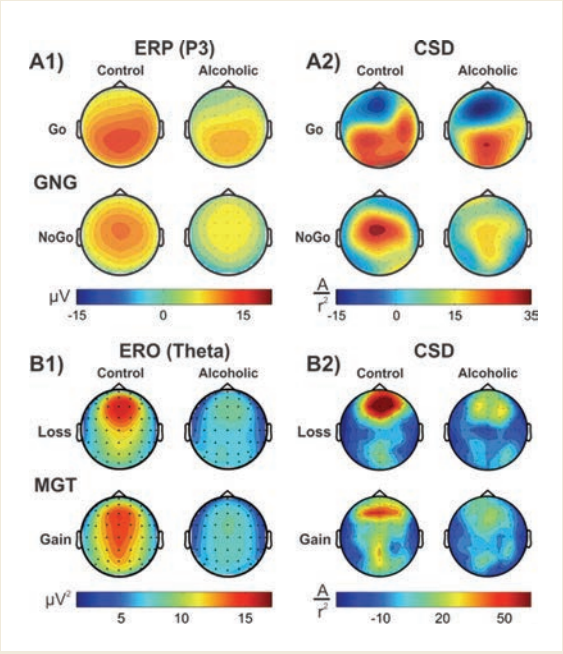

Figure 1 The current source density (CSD) method provides measures of source activations, which are otherwise blurred in the scalp potentials. **A1)** P3 event-related potential (ERP) topography showing lower P3 amplitude (in microvolts) in alcoholics during both Go and No-Go conditions in a Go/No-Go task. **A2)** CSD maps (in ampere per squared radius) showing the Go condition with two bilateral sources in control subjects and only a midline source in alcoholics and illustrating the No-Go condition with a stronger, more focused source over the central region in control subjects and a weaker, more diffuse source over the central and posterior regions in alcoholics (Kamarajan et al. 2005*a*). **B1)** Topography of event-related oscillations (EROs) theta power (in microvolts squared) in alcoholics and control subjects during the loss condition in an monetary gambling tasks (MGT) task, plotted for ERO theta power during the N2-P3 complex (200 to 500 ms). **B2)** CSD maps of ERO theta activity showing a single and stronger midline prefrontal source during the loss condition in control subjects contrasted with bilateral and weaker prefrontal sources in alcoholics; during the gain condition, control subjects had well-defined anterior and posterior sources whereas alcoholics showed weaker and more diffuse sources (Kamarajan et al. 2012).

Componential Analyses of ERPs

PCA

The central idea of the principal component analysis is to reduce the dimensionality of a dataset consisting of a large number of interrelated variables, while retaining as much as possible of the variation present in the dataset. This is done by transforming the data into a new set of variables, called the principal components, which are uncorrelated and often orthogonal and which are ordered so that the first few retain most of the variation present in all of the original variables (Jolliffe 2005). The PCA method decomposes the entire ERP dataset into individual elementary curves or components, and the sum of the derived components should approximate the waveform of the measured ERP (Begleiter et al. 1987*b*). PCA components (i.e., factor loadings or factor waveforms), together with their associated weights (i.e., topography of factor scores), can each be represented in terms of their accounted variance and interpreted based on their topographic significance (Kayser and Tenke 2006). Often, the initially derived components are further subjected to factor rotation (e.g., varimax rotation) to achieve/improve factor structure while maintaining factor orthogonality (being perpendicular from each other) (Kayser and Tenke 2003). Studies have shown that PCA has been useful to segregate components or factors from the ERP data and to determine the dimensionality of effects of interest (Chapman and McCrary 1995; Dien and Frishkoff 2005; Pourtois et al. 2008; Van Boxtel 1998). Performing PCA on the Laplacian transformed waveforms as a generic method for identifying ERP generator patterns also offers unique components with sharper, simpler topographies and

without losing or distorting any effects of interest (Kayser and Tenke 2006). Further, the PCA approach has been applied to decompose time-frequency components of the ERPs to elicit topographically meaningful oscillatory components (Bernat et al. 2005, 2007*b*).

PCA-based decomposition, along with CSD transformation, has been a useful approach to elicit topographically distinct activation patterns to distinguish clinical groups from control subjects, as applied in schizophrenia (Kayser et al. 2006, 2010) and depression (Tenke et al. 2008, 2010). Using a MGT task, Bernat and colleagues (2011) examined the relationship between externalizing proneness and the feedback-related positivity (FRP/P3) and negativity (FRN/N2). Using PCA decomposed time-frequency measures accompanying P3 response to feedback cues revealed that feedback-locked delta-P3 activity was reduced among individuals high in externalizing proneness, whereas theta-N2 response was unrelated to the externalizing index. Begleiter and colleagues (1987*b*) elicited P3 amplitude differences between HR offspring of alcoholics and low-risk control subjects using PCA-derived ERP waveforms. Using a similar method in a flanker task in an alcohol administration study, Bartholow and colleagues (2003) reported that a PCA-derived frontal negativity ERP component was related to the high dose of alcohol during both correct and incorrect response trials. However, incorrect allocation of components and lack of functionally meaningful components have been cited as weaknesses with these methods (Wood and McCarthy 1984), although some solutions have been suggested to overcome these limitations (Dien et al. 2003).

ICA

ICA decomposes ERP data into a set of components that are distinct and maximally independent time courses but are not necessarily orthogonal scalp projections (Makeig et al. 1997). In other words, ICA spatially and temporally filters data without the assumption of the orthogonality of components to represent the input data as a sum of

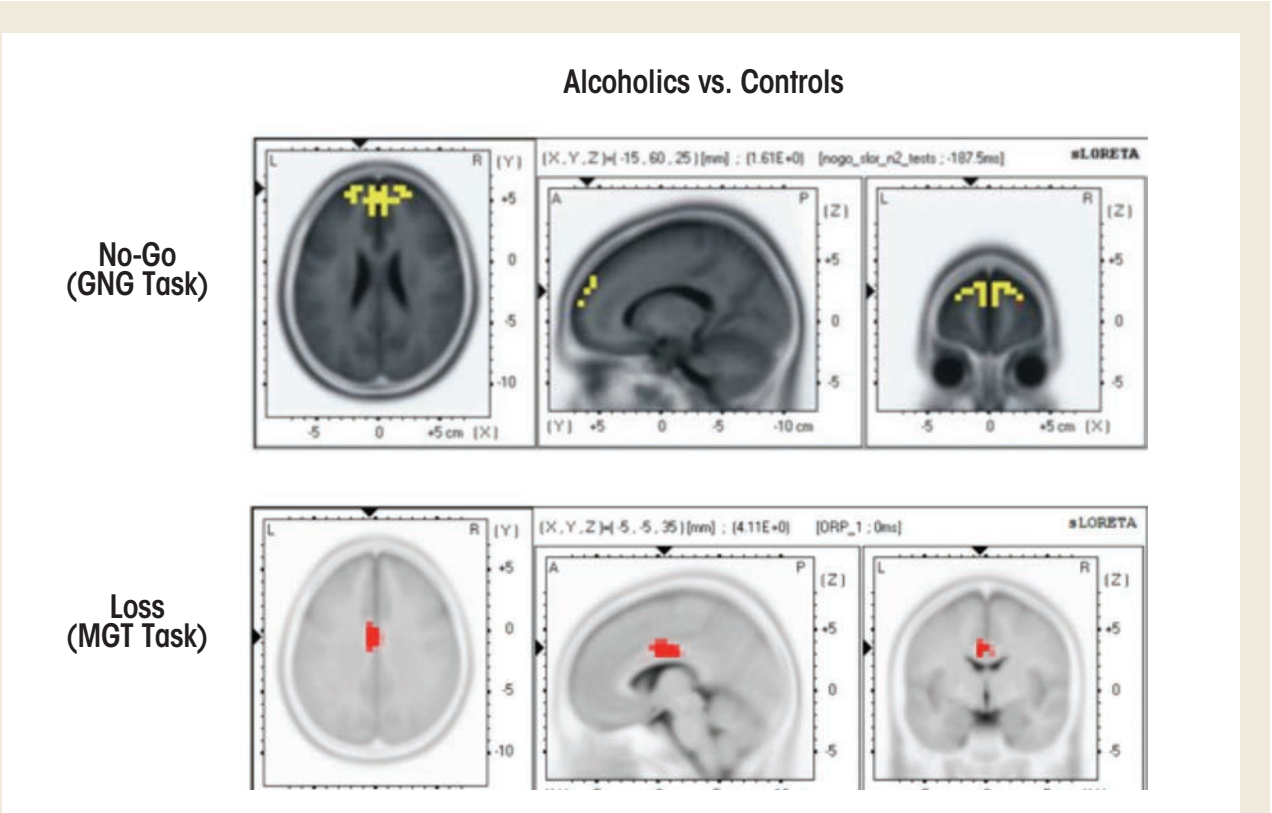

Figure 2 Application of standardized low-resolution brain electromagnetic tomography (sLORETA) to alcoholism. **Top panels:** Current density in alcoholics and control subjects were compared in a Go/No-Go task using sLORETA. Alcoholics showed significantly lower current density activations in bilateral anterior prefrontal regions during No-Go–related N2 activity (yellow blobs in top panels), indicating dysfunctional inhibitory control in alcoholics (Pandey et al. 2012*b*). **Bottom panels:** A sLORETA study in an MGT task found that alcoholics showed decreased current density activation at the middle cingulate cortex region during loss-related P3 activity (red blobs in bottom panels), indicating deficient activation in the reward-related structures or networks (Kamarajan et al. 2010).

temporally independent and spatially fixed components that arise from distinct or overlapping source activations. The ICA method has been demonstrated to extract independent components of early and late ERP potentials that can explain functionally distinct brain processes (Makeig et al. 1999*a,b*), and has been applied to a variety of task paradigms involving perceptual, cognitive and emotional processes (Debener et al. 2005*a*; Desjardins and Segalowitz 2013; Iidaka et al. 2006; Matsumoto et al. 2005; Sato et al. 2001; Schevernels et al. 2014).

Processing steps involved in the derivation of ICA components are illustrated in figure 3, following the method described by Jung and colleagues (2000), visually demonstrating ICA's ability to capture the massive electrooculogram[5] (EOG) activity in the resulting component(s), although its use in decomposing meaningful components underlying ERP components have been illustrated elsewhere (Makeig and Onton 2009; Makeig et al. 1999*a,b,* 2004). These spatially "independent" components are thought to be suggestive of their physiological origins (e.g., eye activity projects mainly from frontal sites and progresses toward posterior sites) (Jung et al. 2001). When these resultant components are combined or "remixed," the original "composite" signal can be obtained.

Whereas some functionally meaningful components help explain the contribution of specific topographic activity

[5] EOG signals are electrical potentials that are generated from movements of the eyeballs, and are measured by pairs of electrodes typically placed above and below or to the left and right of the eye.

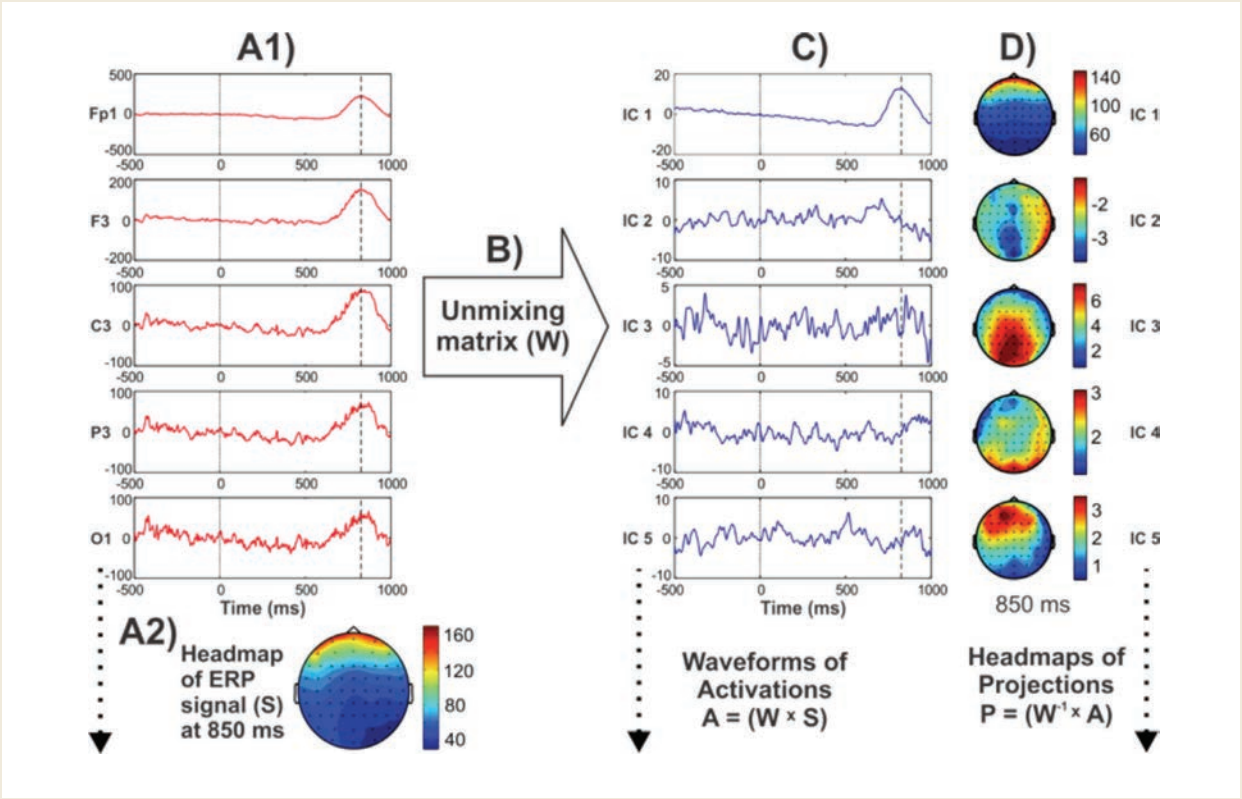

Figure 3 Steps involved in the derivation of independent component analysis (ICA) components in event-related potential (ERP) data, as described by Jung and colleagues (2000, 2001), based on single trials from an ERP dataset from the monetary gambling tasks (MGT) task for illustrative purposes. The waveforms **(panel A1)** and topographic map **(panel A2)** of the ERP signal (S) are shown (in μV) for a trial epoch of an MGT task during the feedback of loss. The "unmixing" matrix (W) **(panel B)** is computed using the ICA algorithm on a "training" dataset (S) representing a larger dataset (e.g., ERP data of adult males during loss condition). "W" consists of weights in a square matrix with the size of number of input channels. The activation matrix **(A)** is obtained by multiplying "W" with "S" **(panel C)**. The rows of "A" represent the time courses of the activations of ICA components. Finally, the "projections" (P) for a given "S" are the product of the inverse matrix of "W" [W-1] and the activations corresponding to the "S" for which ICA components are to be derived **(panel D)**. "P" refers to the relative projection strengths for the respective components at each of the scalp electrodes. It is shown that the EOG activity in the signal (around 850 ms) has been well-captured by the first ICA component. The headmaps have been plotted for 850 ms post-stimulus where the EOG occurs. The 0 (zero) ms on the X-axis of the waveform plots represent the onset of a feedback signal. Downward arrows represent the continuation of the process for remaining electrodes or components.

patterns in the ERP time course (Makeig et al. 1999*a,b*), one or more of the ICA components that are not related to brain processes (i.e., ocular, cardiac, and muscular artifacts) can be removed (Iriarte et al. 2003). ICA algorithms have been used to identify topographic patterns of ERPs associated with specific diagnostic categories, such as mild cognitive impairment (Li et al. 2013; Missonnier et al. 2013*b*) and voluntary hypoxic state (Menicucci et al. 2013). In alcoholism research, Olbrich and colleagues (2002) studied ICA-derived spatial components of ERPs in a visual CNV paradigm and found increases in the ICA components of N2 and negative slow waves as well as decreases in P3 in alcoholics compared with control subjects. Evidence suggests that ICA is becoming a useful signal processing method for analyzing electrophysiological data, and may become an important tool in alcoholism research as well.

Trilinear Modeling

Componential methods such as PCA and ICA estimate the individual spatial and temporal components for a given subject and a given condition separately and do not allow the simultaneous comparison of ERP components across subjects and conditions (Wang et al. 2000). Researchers therefore developed trilinear modeling, a novel method for estimating a set of spatial components (brain maps) and temporal components (waveforms) of time-locked brain potentials across subject groups and task conditions (Wang et al. 2000). Trilinear modeling is one member of a family of modeling techniques that extends two-dimensional linear modeling to multidimensional modeling, in general known as N-way modeling. Trilinear modeling is based on the topographic component model (TCM) (Mocks 1988), which models brain potentials in a trilinear form. The trilinear approach builds on singular value decomposition (SVD) and extends the TCM mainly by replacing the diagonal amplitude matrix by a general loading matrix and by allowing the number of spatial and temporal components to be different (Wang et al. 2000). Thus, the trilinear model has the advantages of both SVD and TCM methods. The trilinear components are uniquely determined and more interpretable. Trilinear modeling can be used for interindividual comparison studies, single-trial modeling, clinical classification of patients, and data filtering. For example, the trilinear method was applied in "dynamic time warping" to align the repeated single trials of the ERPs in order to eliminate the timing differences and to get an improved estimate of the ERP components (Wang et al. 2001). In their original work, Wang and colleagues (2000) had demonstrated the decomposition of visual P3 into 16 spatio-temporal components. Significant linkage between time-warped P3-related trilinear components in a visual oddball paradigm in densely affected alcoholic families from the Collaborative Study on the Genetics of Alcoholism (COGA) has been reported (Porjesz et al. 2002*b*).

The trilinear decomposition method also has been used for resting EEG, to estimate spectral and spatial components. These trilinear components of the resting EEG have been used in a COGA study to reduce multiple testing of electrodes and frequency bands, where significant linkage/linkage disequilibrium and association was found between a trilinear beta EEG phenotype and *GABRA2*, a $GABA_A$ receptor gene, later found to be also associated with alcoholism (Edenberg et al. 2004; Porjesz et al. 2002*a*). (See the section "Electrophysiological Measures as Endophenotypes for Alcoholism.") Trilinear decomposition also has been applied in several studies with EEG (Martinez-Montes et al. 2004; Miwakeichi et al. 2004) and EROs (Morup et al. 2006, 2008). Recently, Verleger and colleagues (2013) applied trilinear decomposition to understand the relationship between CNV and the P3 complex in a Go/No-Go paradigm and obtained relevant components. Trilinear decomposition also has been successfully applied to seizure localization and found to be more sensitive than visual interpretation of the EEGs recorded during a seizure (De Vos et al. 2007). Trilinear modeling has great utility in alcoholism, and further studies are currently being conducted.

EROs: Findings and Prospects

EROs are time-frequency measures of brain electrical activity that are temporally associated with a sensory or cognitive event (Basar et al. 1999, 2001). According to Basar and colleagues (1999), selectively distributed delta, theta, alpha, and gamma oscillatory systems mediate resonant communication networks through large populations of neurons during cognitive processing. The "phase reset model" suggests that ERPs are generated by the resetting of ongoing brain oscillations in response to a neurocognitive event (for a critical discussion, see Sauseng and colleagues 2007). EROs can be classified as (1) "evoked" or phase-locked oscillations, (2) "induced" or non–phase-locked oscillations, and (3) "total" or the summated activity of evoked and induced oscillations (Jones et al. 2006*b*; Tallon-Baudry and Bertrand 1999).

ERO Findings in Alcoholism

EROs provide a useful method to investigate brain dysfunction in alcoholism and risk. Furthermore, they provide powerful quantitative endophenotypes that have been successfully used to identify genes involved in alcoholism (see the section "Electrophysiological Measures as Endophenotypes for Alcoholism"). Several studies have explored EROs in alcoholics as well as HR offspring or relatives of alcoholics, and the key findings are reviewed below.

Delta and Theta EROs

Studies have demonstrated that P3 responses are not unitary phenomena but primarily are the outcome of theta and delta oscillations elicited during stimulus processing (Basar-Eroglu et al. 1992; Karakas et al. 2000*a*,*b*); theta oscillations have a more anterior topography and are maximal

over frontal areas, whereas delta oscillations have a posterior topography and are maximal over parietal areas. ERO delta responses are assumed to mediate signal detection, decision-making, and context/reward processing (Basar 1999*b*; Basar et al. 2001; Kamarajan et al. 2004; Schurmann et al. 2001), whereas ERO theta rhythms are associated with conscious awareness, episodic retrieval, recognition memory, executive control, inhibitory processing, and reward processing (Basar et al. 2001; Doppelmayr et al. 1998; Kamarajan et al. 2004, 2008; Karakas et al. 2000*b*; Klimesch et al. 2001). Studies consistently have found that alcoholics and their HR offspring showed decreased delta and theta ERP power during oddball, Go/No-Go, and MGT tasks (for reviews, see Pandey et al. 2012*a*; Porjesz et al. 2005; Rangaswamy and Porjesz 2008*b*) (see figures 4 and 5).

Gamma Band EROs

Gamma oscillations during cognitive tasks are thought to be involved in selective attention and feature binding (Bertrand and Tallon-Baudry 2000; Fell et al. 2003; Tallon-Baudry et al. 1996). According to Fries and colleagues (2007), gamma rhythm may serve as a fundamental computational mechanism for the implementation of a temporal coding scheme that enables fast processing and flexible routing of activity during signal processing, by supporting fast selection and binding of distributed responses. Particularly, early phase-locked gamma is involved in the selection/identification of target stimuli and represents top-down mechanisms during selective attention (Fell et al. 2003). Neuroimaging studies have identified fronto-parietal attentional networks that may subserve the top-down control of

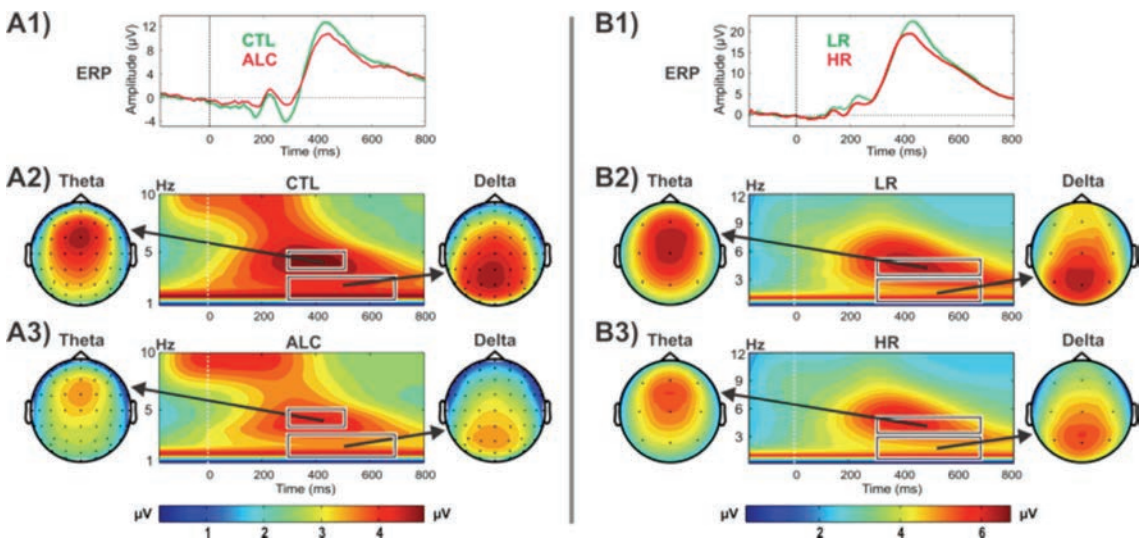

Figure 4 Application of event-related potentials (ERPs) and event-related oscillations (EROs) in alcoholism during a visual oddball task (Jones et al. 2006*b*; Rangaswamy et al. 2007). The left side of the figure **(panels A1–A3)** compares alcoholics (ALC) and control subjects (CTL) (Jones et al. 2006*b*), whereas the right side of the figure **(panels B1–B3)** compares high-risk (HR) offspring and low-risk (LR) control subjects (Rangaswamy et al. 2007). Alcoholics showed lower P3 amplitudes than control subjects **(panel A1)**, whereas HR offspring showed lower P3 amplitudes to targets than LR in the same visual oddball paradigm **(panel B1)**. Panel A2 illustrates time-frequency (TF) plots for control subjects (center rectangular panel) with accompanying topographical head plots for delta (1 to 3 Hz) at the Pz electrode (right) and theta (4 to 5 Hz) at the Fz electrode (left). **Panel A3** illustrates corresponding TF plots for alcoholics (center rectangular panel) with accompanying topographical head plots for delta (right) and theta (left). Alcoholics showed lower activation of both delta and theta EROs compared with control subjects **(panels A2–A3)** during the processing of targets. **Panel B2** illustrates TF plots for LR (center rectangular panel) with accompanying topographical head plots for delta (1 to 3 Hz) at the Pz electrode (right) and theta (4 to 5 Hz) at the Fz electrode (left). **Panel B3** illustrates corresponding TF plots for HR (center rectangular panel) with accompanying topographical head plots for delta (right) and theta (left). Similar to the alcoholics, HR offspring manifested lower activation in P3 **(panel B1)**, delta and theta EROs (**panels B2–B3)** compared with LR control subjects.

selective attention (Corbetta et al. 2000; Giesbrecht et al. 2003). This early evoked gamma activity has been reported to be larger to attended (target) compared with unattended (non-target) stimuli, suggesting a top-down control mechanism (Busch et al. 2006; Debener et al. 2003; Yordanova et al. 2002).

Studies have found that early evoked gamma activity was abnormal (either higher or lower) in patients with psychiatric disorders (e.g., Basar-Eroglu et al. 2007; Ozerdem et al. 2010; Yordanova et al. 2001). In abstinent alcoholics, researchers have reported a significantly reduced gamma band (28 to 45 Hz) response (0 to 150 ms) in the frontal region during target stimulus processing in a visual oddball task (Padmanabhapillai et al. 2006*a*). Similar reductions in early gamma response also have been found in children of alcoholics (ages 7–17 years) at the posterior regions (Padmanabhapillai et al. 2006*b*). The regional variation in gamma differences observed in children of alcoholics

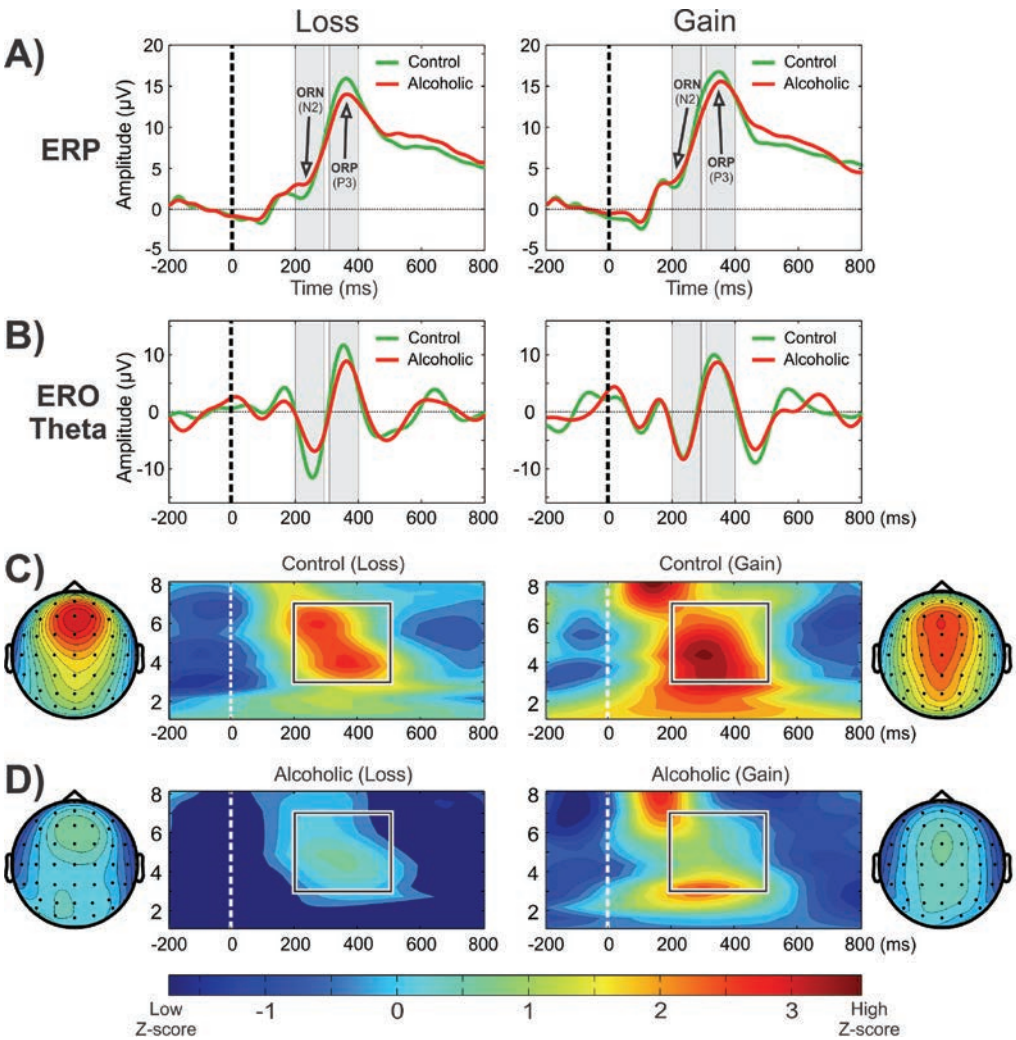

Figure 5 Application of event-related potentials (ERPs) and event-related oscillations (EROs) to alcoholism in a monetary gambling task (MGT) (Kamarajan et al. 2012). **A)** Alcoholics showed lower P3 amplitude of the ERP during loss and gain conditions than control subjects. **B)** ERO theta activity (3 to 7 Hz) was lower during the N2 and P3 time window in alcoholics compared with control subjects. **C)** Time-frequency plots (center panel) and topographic head plots of theta power in control subjects during loss (left) and gain (right) conditions. **D)** Time-frequency plots (center panel) and topographic head plots of theta power in alcoholics during loss (left) and gain (right) conditions. Theta power was lower in alcoholics during loss and gain conditions compared with control subjects

compared with adult alcoholics could be attributed to the fact that the frontal lobes still are in the process of maturation in children and adolescents (Sowell et al. 2004). These deficits further emphasize the view that alcoholism may be associated with deficient frontal (top-down) processing and a dysfunctional fronto-parietal attentional network (Goldstein and Volkow 2011; Rangaswamy et al. 2004*a*).

Advances in ERO Methods

Event-Related Desynchronization and Synchronization (ERD/ERS)

ERD/ERS is a valuable technique to unravel time–frequency–space dynamics of cortical oscillations across brain regions during cognitive and motor processing (Klimesch et al. 1997; Krause 2006; Pfurtscheller 1999, 2001; Pfurtscheller and Aranibar 1979). According to Pfurtscheller (2001), ERD represents an activated cortical area with increased excitability, whereas ERS indicates a deactivated cortical area with decreased excitability. Specifically, ERD represents the percentage of decrease, whereas ERS indicates an increase in band power during an event as compared with power in a baseline window (Doppelmayr et al. 1998).

The ERD/ERS method has been useful in understanding cognitive processing abnormalities in several clinical conditions, such as schizophrenia (Bachman et al. 2008; Fujimoto et al. 2012; Xu et al. 2013), attention-deficit hyperactivity disorder (Missonnier et al. 2013*a*), Alzheimer's disease (Babiloni et al. 2000; Karrasch et al. 2006), Parkinson's disease (Dushanova et al. 2010; Ellfolk et al. 2006; Labyt et al. 2003), and epilepsy (Houdayer et al. 2012; Pfurtscheller et al. 2003; Visani et al. 2011). A few studies have investigated the acute effects of alcohol on brain oscillatory responses. Krause and colleagues (2002) studied alcohol-induced alterations in ERD/ERS during an auditory memory task and found that alcohol decreases alpha-ERS responses during encoding and increases alpha-ERD responses during recognition. In an alcohol-approach avoidance task, Korucuoglu and colleagues (2014) found that acute alcohol facilitates response preparatory processes for approach alcohol trials in social drinkers. Posterior beta-ERD was found to increase during preparation for alcohol-approach trials, whereas the beta-ERD in the congruent block increased following alcohol administration. Studies using ERD/ERS measures in alcoholism are currently being conducted.

Connectivity Measures During Task-Related Conditions

ERO Coherence

Coherence is an estimate of the consistency of relative amplitude and phase between two signals within a frequency band and represents functional interactions across brain regions (see the earlier section "Resting EEG Coherence"). When this coherence function is measured with the same algorithm but using signal processing techniques to extract time-frequency measures (e.g., EROs with S-transform, matching pursuit, wavelet transform, etc.) during a cognitive task, it represents functional connections between neural systems associated with specific cognitive activity (Qassim et al. 2013; Sakkalis 2011). This linear coherence measure generally is distinct from phase synchronization or phase synchrony (Lachaux et al. 1999), which refers to the method that measures phase locking (i.e., level of phase alignment) between signals oscillating at the same frequency (see the next section for details). Thus, ERO coherence is a linear function computed instantaneously by applying time-frequency analysis, such as wavelet analysis, to activity during a task (Torrence and Compo 1998). Using the coherence method, studies have identified possible dysfunction in connectivity between brain regions in several neuropsychiatric conditions (for reviews, see Basar 2013; Sakkalis 2011; Yener and Basar 2013). Diminished event-related gamma band coherence has been reported in schizophrenia (Sakkalis et al. 2006) and bipolar disorder (Ozerdem et al. 2011). In alcoholics, a recent study (Ismaili et al. 2012) found significantly increased wavelet coherence in theta (4 to 8 Hz), alpha (8 to 13 Hz), and gamma (50 to 60 Hz) bands at frontal and occipital regions during 100 to 200 ms poststimulus while performing a visual discrimination task. More alcoholism studies applying this method are under way.

ERO Phase Synchronization

Phase synchronization is a measure of phase locking between two signals (Lachaux et al. 1999) and represents a mechanism for long-range neural integration involving interactions between the participating local networks (Varela et al. 2001). In event-related data, phase synchronization quantifies the phase differences between the signals across trials (phase-locking factor) by extracting the instantaneous phase of each signal at the specified (target) frequency (Lachaux et al. 1999). Phase-locking factor (also called intertrial phase coherence) is a measure of phase consistency across trials from a single electrode or source (Delorme and Makeig 2004). The phase synchronization method assumes that two dynamic systems may have their phases synchronized, even if their amplitudes are zero correlated (Mormann et al. 2000; Sakkalis 2011). Thus, phase synchronization measures the similarity of two time series (signals) in terms of phase consistency or phase-locking factor and varies in value between 0 (no synchronization) to 1 (perfect synchronization) (Lachaux et al. 1999; Tallon-Baudry et al. 1996). During the processing of cognitive tasks, the phase-locking index varies based on task conditions, brain regions, and frequency bands. For example, Kolev and colleagues (2001) investigated phase locking during passive listening to repeated stimuli and active counting of target stimuli and found condition-specific phase-locking indices of alpha

oscillations. Similarly, using a Go/No-Go task, Muller and Anokhin (2012) reported that the phase-locking index and phase synchronization were the highest in the Go and No-Go conditions, intermediate in the warning condition, and the lowest in the neutral condition of the task and elicited distinct, dynamic functional networks for response inhibition and execution.

Although the linear coherence measure does not separate the effects of amplitude and phase in the interrelations between the signals, phase synchronization also yields the phase information, which is important to understand the event-related brain dynamics (Lachaux et al. 1999). Dysfunction in phase synchronization during information processing has been reported in several clinical conditions (for a review, see Uhlhaas and Singer 2006), such as schizophrenia (Csukly et al. 2014; Griesmayr et al. 2014; Perez et al. 2013), depression (Olbrich et al. 2014), obsessive compulsive-disorder (Olbrich et al. 2013), and externalizing disorders (antisocial behavior, attention deficit hyperactivity disorder, and substance dependence) (Burwell et al. 2014). In alcoholism, Sakkalis and colleagues (2007) reported that alcoholics showed impaired synchronization and loss of lateralization, most prominently in alpha- and lower beta–frequency bands, during mental rehearsal of pictures. Studies are under way to elucidate further oscillatory dynamics underlying cognitive (dys)function in alcoholics and in HR subjects.

Granger Causality Analysis

When applied to brain signals, Granger causality as a statistical method measures the degree of predictability of temporal changes in one brain region that can be attributed to those in another region (Bressler and Menon 2010). According to Granger (1969), causal influence can be explained in terms of stochastic (random) processes when the predictability of one process at a given time point is improved by including measurements from the other. Whereas the coherence methods yield only the strength (but not the direction) of the connection, Granger causality can show both strength of connection and directionality for stationary signals. Thus, this method is suitable for the study of directional influences and pathways in neural networks using both frequency and time domains of ERO data (cf. Brovelli et al. 2004).

Granger causality has been successfully used to identify coupling (connectivity) and information exchange across brain regions in a variety of clinical conditions, such as developmental dyslexia (Ligges et al. 2010), epilepsy (Adhikari et al. 2013; Chavez et al. 2003), and Alzheimer's disease (Dauwels et al. 2009, 2010). Studies are being conducted using Granger causality to understand the directionality of the neural pathways across brain regions involved in neural processing in alcoholics and their HR offspring.

Potential for Translational Applications of Electrophysiological Measures of Brain Function

Electrophysiological measures and techniques have clinical applications in several important areas, including genetics/endophenotypes, and to inform the fields of diagnostic classification, prevention, response to treatment, cognitive remediation, neurofeedback, and deep brain stimulation (DBS). Current clinical applications and future translational potential of electrophysiological assessments, especially in the context of alcoholism, are discussed below.

Electrophysiological Measures As Endophenotypes for Alcoholism

Risk for alcoholism is complex and influenced by both genetic and environmental influences and their interactions: multiple genes, each with small effect, phenotypic complexity and heterogeneity, environmental variability, gene–gene interactions, and gene-by-environment interactions (Porjesz and Rangaswamy 2007). It is difficult to find genes affecting complex diseases such as alcoholism and to use diagnosis as the sole phenotype (Tsuang and Faraone 2000). One effective strategy to find genes is the "endophenotype" approach, first proposed by Gottesman and Shields (1972), who defined an endophenotype as an intermediary measure of neuropsychiatric functioning correlated with the main trait of interest and involved in the pathway between genotype and outcome of interest (Gottesman and Gould 2003). An effective endophenotype must meet three important criteria: (1) it is associated with the illness in the population (i.e., present in affected individuals); (2) it is heritable; and (3) it is found in unaffected relatives of probands at a higher rate than in the general population (including offspring before the onset of the illness). Neurophysiological quantitative measures that meet these three criteria can serve as effective endophenotypes. That is, they can help identify genes associated with the disorder and elucidate mechanisms that may improve understanding of the disorder. Specifically, only heritable electrophysiological measures that differentiate alcoholics from nonalcoholics are used as endophenotypes, to be sure that the measure is related to the disorder (alcoholism). Furthermore, the neurophysiological measure must be able to differentiate between HR offspring of alcoholics and low risk offspring of non-alcoholics (controls) who have no first or second degree alcoholic relatives, and are not at high risk to develop alcoholism (Porjesz and Rangaswamy 2007). These highly heritable and quantitative measures are closer to the gene function, and several measures (e.g., beta power and theta coherence of resting EEG, P3 amplitude and related theta and delta EROs during the oddball task) have been successfully used to identify genes associated with risk for alcoholism and related disorders (for reviews, see Porjesz and Rangaswamy 2007; Rangaswamy and Porjesz 2008*a*,*b*).

One EEG measure, the beta rhythm (i.e., beta 1 [12.5 to 16 Hz], beta 2 [16.5 to 20 Hz], and beta 3 [20.5 to 28 Hz]

bands) of the resting EEG, meets criteria as an endophenotype. Beta power is highly heritable (86 percent) (van Beijsterveldt et al. 1996) and is increased in alcoholics (e.g., Bauer 2001; Rangaswamy et al. 2002) and HR offspring (e.g., Pollock et al. 1995; Rangaswamy et al. 2004*b*). Enoch and colleagues (2003) found that LVA in female subjects was associated with a genetic variant that leads to low activity in COMT, the enzyme that metabolizes dopamine and norepinephrine (NE), leading the researchers to hypothesize that altered NE levels may be related to LVA, anxiety, and alcoholism. Beta power has been found to have a genetic link and association with *GABRA2*, a receptor gene for $GABA_A$ (Edenberg et al. 2004; Porjesz et al. 2002*a*). Beta rhythm is attributed to a balance between networks of excitatory pyramidal cells and inhibitory interneurons involving $GABA_A$ action as the pacemaker (Whittington et al. 2000). The increased beta in alcoholics and HR offspring indicates an imbalance in excitation-inhibition (CNS disinhibition) that precedes the development of alcoholism and may be an index of a predisposition to it (Porjesz et al. 2005). Association of the *GABRA2* receptor gene with a diagnosis of alcohol dependence originally was reported in the COGA study (Edenberg et al. 2004) and replicated by many other studies worldwide (Covault et al. 2004; Fehr et al. 2006; Lappalainen et al. 2005; Philibert et al. 2009; Soyka et al. 2008). In COGA, it has been found that the association with *GABRA2* in adults was strongest in alcoholics who were more severely affected and in those who also had comorbid SUDs (Agrawal et al. 2006). In children, *GABRA2* was found to be associated with conduct disorder, a precursor phenotype (Dick et al. 2006). The heritability of EEG coherence has been examined in twin and family studies (Chorlian et al. 2007; van Baal et al. 2001; van Beijsterveldt et al. 1998). Further, in COGA, a high theta-coherence phenotype has been found to be linked and associated with two inhibitory neurotransmitter receptor genes: *GABRA2*, and *CHRM2*, a muscarinic acetylcholine receptor gene (Porjesz and Rangaswamy 2007; Rangaswamy and Porjesz 2008*a*). Taken together, this endophenotype approach presents a biological hypothesis relating underlying CNS disinhibition to a genetic risk for alcoholism and related disorders. Variations in $GABA_A$ receptor genes influence neural excitability and an imbalance in excitation-inhibition, manifesting as increased beta activity (hyperexcitability or CNS disinhibition) in alcoholics and HR offspring, which in turn may be involved in the predisposition to develop AUD and related disinhibitory disorders. This supports the hypothesis originally proposed by Begleiter and Porjesz (1999).

In addition to resting-state EEG endophenotypes, P3-related measures during cognitive tasks (e.g., oddball task) have been successfully used as endophenotypes to identify genes related to alcoholism. Chen and colleagues (2010) reported significant associations between the P3 amplitude to visual targets as well as to alcohol dependence diagnosis with multiple single nucleotide polymorphisms (SNPs) in the corticotrophin-releasing hormone receptor 1 (*CRHR1*) gene, which has been shown to have a role in the environmental stress response in ethanol self-administration animal models.

One neurophysiological measure that has been successfully used as an endophenotype in identifying several genes associated with alcoholism is the frontal theta ERO during P3 to targets during a visual oddball task in COGA (Chen et al. 2009; Jones et al. 2004, 2006*a*; Kang et al. 2012; Zlojutro et al. 2011). Genetic linkage and association with a muscarinic acetylcholine receptor M2 (*CHRM2*) and frontal theta and posterior delta EROs underlying P3 were reported (Jones et al. 2004, 2006*a*). SNPs in *CHRM2* have been found with comorbid alcohol dependence and depression (Wang et al. 2004) and comorbid alcohol and drug dependence (Dick et al. 2007). Significant linkage and association were reported for the *CHRM2* gene and a spectrum of externalizing disorders in the COGA study (Dick et al. 2008). Luo and colleagues (2005) replicated these findings of an association with *CHRM2*, for alcohol dependence, drug dependence, and affective disorder. Significant linkage and association with *CHRM2* also was found with high theta (6 to 7 Hz) interhemispheric coherence. This high theta interhemispheric coherence also was linked and associated with *GABRA2*. Both GABAergic and cholinergic systems are important in local inhibitory circuits essential for cortical synchronization in the theta band (Porjesz and Rangaswamy 2007). M2 receptors are concentrated in the forebrain and have an inhibitory role in the generation of theta and delta EROs via inhibition of presynaptic release of acetylcholine (Frodl-Bauch et al. 1999). P3 production requires both inhibition of irrelevant networks and activation of relevant ones, and it is likely that *CHRM2* affects the inhibition of irrelevant networks during P3 tasks. In Alzheimer's disease, where there is degeneration of cholinergic neurons in the nucleus basalis, abnormal theta delta and P3 have been reported. Results from the COGA study showed that delta EROs and *CHRM2* affect the onset of regular alcohol use and alcohol dependence during adolescence and young adulthood (Chorlian et al. 2013). Hill and colleagues (2013) used group-based trajectory modeling of auditory P3 data collected longitudinally from offspring in families with and without familial risk for AUD and found that specific trajectories of P3 were associated with familial risk and *CHRM2* variation, with high familial risk in male offspring. These findings underscore the utility of P3-related measures as effective endophenotypes in genetic studies of psychiatric disorders.

Under the same linkage peak as the *CHRM2* gene, a metabotropic glutamate receptor gene (GRM8) was found to be associated with theta EROs to target stimuli at frontal, central, and parietal regions. The same SNPs were found to be significantly associated with 1CD-10 (World Health Organization 1992) based alcohol dependence (Chen et al. 2009). The neurochemical basis of the target stimulus response—P3 and related theta and delta rhythms—is

triggered by glutamatergic activity and modulated by both cholinergic and GABAergic sources (Frodl-Bauch et al. 1999). These same GABAergic, cholinergic, and glutamatergic receptor genes also were found to be associated with alcoholism-related phenotypes. Thus, the same genes initially identified as associated with electrophysiological endophenotypes also were found to be associated with alcoholism-related phenotypes.

In a family-based genome-wide association study, Kang and colleagues (2012) used the same neurophysiological phenotype (frontal theta ERO in the visual oddball task) and found genome-wide significant association with several SNPs in KCNJ6, a gene that encodes the protein G-protein inward-rectifying potassium channel 2 (GIRK2). GIRK2 is widely distributed in the brain and is important in dopaminergic, cholinergic, GABAergic, and glutamatergic synapses (Saenz del Burgo et al. 2008). GIRK2-receptor activation contributes to slow inhibitory postsynaptic potentials that modulate neuronal excitability and therefore is important in regulating excitability of neuronal networks. GIRK2 also is important in alcoholism studies, as it is directly activated by alcohol (Aryal et al. 2009; Blednov et al. 2001; Bodhinathan and Slesinger 2013; Hill et al. 2003; Kobayashi et al. 1999; Lewohl et al. 1999). In addition, GIRK2 receptors are important effectors in both opioid- and alcohol-induced pain relief (Ikeda et al. 2002) and are viable drug targets (Kobayashi et al. 2004; Lotsch and Geisslinger 2011).

These findings further underscore the utility of electrophysiological and neurogenetics in understanding the genetics of alcoholism. Recent and future advances in genetic technology hold promise to enhance our understanding of the pathophysiology of AUD as well as to identify potential targets (e.g., neurotransmitter systems and pathways) for drug discovery for prevention and treatment of alcoholism and related disorders (Bodhinathan and Slesinger 2014).

Clinical or Translational Aspects of Electrophysiological Measures of Brain Function

Diagnostic Classification and Subtyping

Quantitative electrophysiological measures have been used to classify patients into diagnostic categories and to identify subtypes within a diagnostic category (Bernat et al. 2007*a*; John et al. 2007; Karaaslan et al. 2003; Prichep et al. 2002). In alcoholism, Branchey and colleagues (1988) found that decrements in P3 amplitude characterized a subgroup of alcoholics with disordered regulation of aggression. Bauer (1994) reported that resting EEG absolute beta power (13.2 to 27.6 Hz) at the vertex (Cz electrode) was observed more in relapse-prone patients than in abstinence-prone patients and control subjects. Bauer (1997) also found that P3 could discriminate multiple subgroups within alcoholism: (1) there was more reduction in visual P3 amplitudes at frontal electrode sites among patients with antisocial personality disorder (ASPD), relative to ASPD-negative patient and control groups; (2) the frontal P3 decrement was significantly correlated with the number of childhood conduct disorder symptoms but not with the presence/absence of a family history of alcoholism; and (3) discriminant function analysis revealed that P3 amplitude alone accurately identified 70.6 percent of the patients who later relapsed and 53.3 percent of the patients who did not. Hegerl and colleagues (1995) recorded N1 and P2 components to auditory stimuli in five different intensities in hospitalized alcoholic patients after 1 week of withdrawal and found that patients with antisocial tendencies showed a significantly stronger intensity dependence of their evoked responses of primary auditory cortices (tangential dipoles). This suggests that alcoholics with strong intensity dependence in their ERPs, along with antisocial tendencies, formed a subgroup with a serotonergic hypofunction and may respond favorably to relapse prevention with serotonergic drugs. Furthermore, Winterer and colleagues (2003) found that increases in bilateral, intrahemispheric posterior coherences in the alpha and beta frequency in alcohol-dependent study participants covaried with depressiveness. Taken together, these findings suggest that electrophysiological measures of brain function can aid in diagnostic classification and subtyping, which may lead to better prevention and treatment strategies.

Prevention, Response to Treatment or Medications

Neurophysiological measures can potentially aid in prevention strategies. In a comprehensive review on trait markers for alcoholism, Farren and Tipton (1999) offer the possibility that electrophysiological markers, such as low EEG response to alcohol (e.g., Volavka et al. 1996) and reduced P3 wave (Porjesz et al. 2005), are good predictors of the development of later substance abuse in predisposed youths. The authors suggest that these measures therefore are potentially viable tools for identifying subgroups of vulnerable individuals and might be implemented in alcoholism prevention programs. Some of these electrophysiological measures have offered established tools to compare clinical outcomes, such as response to medication or treatment, in several psychiatric disorders (Hegerl and Herrmann 1990; Prichep and John 1992; Suffin and Emory 1995), including schizophrenia (Knott et al. 2000), depression (Bruder et al. 2008, 2013; Cook et al. 2005), attention-deficit hyperactivity disorder (Arns et al. 2008; Chabot et al. 1999), and alcoholism (Cristini et al. 2003; Saletu-Zyhlarz et al. 2004). For example, Ford and colleagues (1986) measured EEG coherence in individuals with paranoid schizophrenia, dysthymia, and affective disorder who received tricyclics, neuroleptics, or no medication and found that coherence values were highest in paranoid schizophrenics, decreased with neuroleptic medication, and increased with tricyclic antidepressants. Similarly, Hegerl and colleagues (1992) found

that responders to prophylactic lithium medication in affective psychosis showed a steeper slope of the amplitude/stimulus-intensity function (ASF slope) in N1 and P2 components than in nonresponders. The pronounced amplitude increases in the Loudness Dependence of the Auditory Evoked Potential (LDAEP), such as tangential dipole activity and N1-P2 components with increasing stimulus intensity (loudness), have been proposed as an indicator of a low serotonergic neurotransmission. This feature of augmented LDAEP has been observed during the alcohol-intoxicated state (Hegerl et al. 1996*a*) and after the intake of acamprosate during treatment (Hegerl et al. 1996*b*). Further, Pillay and colleagues (1996) showed that female patients with abnormal EEGs before starting the clozapine treatment had a significantly greater improvement in global assessment of functioning scores compared with female patients with normal EEGs. These results suggest that electrophysiological measures are useful to predict a clinical response in specific groups of patients.

In alcoholism, Cristini and colleagues (2003) found that the P300 amplitudes to targets in an auditory oddball paradigm as well as in a CNV paradigm were significantly higher among patients who relapsed during the 3-month follow-up than in those who remained abstinent. Saletu-Zyhlarz and colleagues (2004) compared EEG profiles of relapsing patients with those of abstaining patients during 6 months of pharmacologically supported relapse prevention therapy. Aberrant brain function, characterized by a decrease in delta and slow alpha power and an increase in beta power, was more pronounced in relapsing than in abstaining patients. Further, after 6 months of treatment, only the abstaining patients showed an increase in slow activity, a decrease in fast alpha, an acceleration of the delta/theta centroid, and a deceleration of the alpha centroid, reflecting a normalization of brain function. These findings suggest that EEG measures may serve as useful prognostic indicators in alcoholism. However, notwithstanding the proven and potential applications, electrophysiological tools are often thought to have not yet been optimized as standardized outcome measures for their use in clinical trials (Cho et al. 2005).

Cognitive Remediation Techniques

Cognitive remediation is a neurobehavioral treatment that uses repetitive practice and compensatory and adaptive strategies to facilitate improvement in targeted cognitive areas, such as memory, attention, and problem solving (Medalia and Choi 2009). Cognitive remediation, also called "cognitive retraining" or "cognitive rehabilitation," has been applied to several neurological and psychiatric conditions (for a review, see Langenbahn et al. 2013), including alcoholism (Allen et al. 1997; Godfrey and Knight 1985; McCrady and Smith 1986). Electrophysiological measures can serve as metrics of cognitive functioning during pre- and posttreatment of cognitive remediation. For example, Horowitz-Kraus and Breznitz (2009) found that brain activity changed in dyslexic patients as a result of working-memory training, as evidenced by an increase in both working-memory capacity and the amplitude of the ERN component of the ERPs. When ERN amplitudes increased, the percentage of errors on the Sternberg test of working memory decreased, suggesting that by expanding the working-memory capacity, larger units of information are retained in the system, enabling more effective error detection.

According to Campanella and colleagues (2011), electrophysiological methods can guide the clinician to optimize the medication regimen tailored to a patient's cognitive profile and adopt a kind of "personalized medicine" (Campanella et al. 2011). For example, alcoholic patients with attentional biases toward alcohol cues (indexed by increased P100 to probes replacing drug cues), but with intact inhibitory processes (indexed by normal No-Go P3 component), may hypothetically benefit more from acamprosate (which regulates the increased cerebral glutamate activity by restoring the balance between excitatory and inhibitory neurotransmission), by reducing the hyperexcitability that occurs during early abstinence. However, alcoholic patients with a reversed cognitive pattern (i.e., a deficient inhibitory mechanism with altered No-Go P3 but without any attentional biases toward alcohol cues indexed by normal P100) will likely improve with naltrexone (an opioid antagonist which blocks the release of alcohol-induced dopamine), which reduces or eliminates the positive reactions associated with the urge to drink and inhibits a dominant response (drinking) by reducing the reinforcing/reward effects of alcohol (Campanella et al. 2011). Although these hypothesized applications are intriguing, more studies are needed to find empirical support for the possible role of electrophysiological measures in this type of personalized medicine; caution is suggested for any direct clinical application based on these findings and their implications, as more empirical evidence is still needed.

Neurofeedback

The treatment of addictive disorders by EEG biofeedback (or neurofeedback, as it often is called) was first popularized by the work of Eugene Peniston (Peniston and Kulkosky 1989, 1990, 1991; Saxby and Peniston 1995) and became popularly known as the Peniston Protocol. This approach employed independent auditory feedback of two slow brain-wave frequencies, alpha (8 to 13 Hz) and theta (4 to 8 Hz) in an eyes-closed condition to produce a hypnagogic state. Patients were taught before neurofeedback to use "success imagery" (being sober, refusing offers of alcohol, living confidently, and being happy) as they drifted down into an alpha-theta state. Repeated sessions reportedly resulted in long-term abstinence and changes in personality (cf. Sokhadze et al. 2008). Several studies have reported that

the Peniston neurofeedback method has been effective in achieving abstinence and improving cognitive and behavioral symptoms (cf. Saxby and Peniston 1995). For example, Saxby and Peniston (1995) reported that only 1 of 14 patients had relapsed by 21 months after neurofeedback training.

Compared with a nonalcoholic control group and a traditionally treated alcoholic control group, alcoholics who received brainwave training showed significant increases in percentages of alpha and theta rhythms in the EEG traces (as visually assessed by blind raters), increased amplitude in alpha rhythm, and sharp reductions in depression scores compared with the control groups (Peniston and Kulkosky 1989). Neurofeedback techniques have been found to be effective for treating alcohol and other SUDs (Sokhadze et al. 2008; Trudeau et al. 2009) and to improve performance and well-being in individuals with other behavioral/emotional problems (Gruzelier 2009). According to Gruzelier (2009), neuroanatomical circuitry underlying alpha-theta neurofeedback involves cognitive as well as affective/motivational functions subserved by the interaction between distal and widely distributed brain connections, mainly from the ascending mescencephalic-cortical arousal system and limbic circuits. These studies suggest that neurofeedback methods may become effective therapeutic tools for AUD, although more studies are needed to both confirm and enhance their applications.

DBS

Another potential area for the application of electrophysiological measures in the treatment of addiction is DBS (Kuhn et al. 2011, 2013; Voges et al. 2013). DBS involves electrical stimulation of high-frequency electrodes surgically placed in one or more specific brain region(s), including the ventral intermediate nucleus of the thalamus, the subthalamic nucleus, and the internal segment of the globus pallidus. This technique is aimed at ameliorating the symptoms of movements, cognition, and emotions in several neuropsychiatric conditions (Luigjes et al. 2013; Perlmutter and Mink 2006). As a result of its successful application and approval for several neurological disorders, DBS is thought to be a powerful tool for modulating dysregulated networks and also has been considered for treating substance addiction (cf. Kuhn et al. 2013). DBS is a surgical procedure performed in the treatment/rehabilitation of some neurological conditions (Lyons 2011) and SUDs (Kuhn et al. 2013; Munte et al. 2013). Although electrophysiological measures do not have any direct role in this neurosurgical procedure, per se, they can aid in followup and maintenance of cognitive functioning in patients with DBS. For example, Kuhn and colleagues (2011) assessed cognitive control using psychometric and electrophysiological measures in severely alcohol-dependent patients who recently had undergone DBS procedures for addiction treatment and found that DBS drastically reduced addictive behavior and craving. Further, error-related negativity, an electrophysiological marker of error processing linked to anterior mid-cingulate cortex functioning, was altered after the DBS surgery, an effect that could be reversed by periods without stimulation. This case illustrates the utility of electrophysiological measures to aid in the follow-up treatment in DBS. Fins and Shapiro (2014) suggest that brain-mapping methods may advance the potential applications of DBS in the perspective of personalized medicine. However, further electrophysiological research is warranted to understand and optimize the effectiveness and outcome of this potentially promising method.

Summary and Future Directions

This article has summarized and discussed several electrophysiological measures and tools available for alcoholism research. (See the accompanying table for a summary of findings for each method and measure.) Although advances in several electrophysiological methods are highlighted, some of these newer techniques have not yet been used to explore AUD. Nevertheless, many of these tools have potential for applications to characterize and understand alcoholism and other related disorders, and recommendations have been made to apply these novel tools or techniques to the field of alcoholism. Further, the translational potential for electrophysiological measures of brain function as endophenotypes and as valuable tools to aid in prevention, diagnosis, treatment, and rehabilitation have been briefly discussed.

Future research will focus on newer and more effective electrophysiological techniques available for neurocognitive, genetic, and clinical research. Given that electrophysiological measures hold promise as effective endophenotypes for gene discovery, these tools have potential for drug discovery as well as for a range of clinical applications. A variety of sophisticated statistical techniques (e.g., developmental trajectories), which will allow systematic research on several key electrophysiological measures, will be useful to highlight longitudinal aspects of cognitive development as well as the nature and course of the disorder under investigation. Furthermore, recent research has capitalized on the potential of using complementary information from neuroimaging methods and electrophysiological measures by performing multimodal brain imaging (Uludag and Roebroeck 2014) (e.g., combined EEG–fMRI studies), which has offered and will offer remarkable findings to understand the disorder in a better light than any single method can potentially promise (He and Liu 2008). The studies using multimodal imaging approaches are growing rapidly by implementing a simultaneous EEG–fMRI protocol aimed at achieving both high temporal and spatial resolution of human brain function (Huster et al. 2012; Mantini et al. 2010), and this approach already has been found to be highly useful in many clinical conditions (Gotman and Pittau 2011; Shafi et al. 2012), including alcoholism (De Ridder et al. 2011; Karch et al. 2008). As a final note, as advancement in tech-

Table Summary of Major Electrophysiological Findings in Alcoholism

Method/Measure	Function/Dysfunction	Findings in Alcoholics	Findings in High-Risk (HR) Offspring/Relatives
Resting electroencephalogram (EEG): delta power (1 to 3 Hz)	Integration of cerebral activity with homeostatic processes. Increased awake delta power is related to neurological and psychiatric conditions.	Equivocal (both increase and decrease reported).	No significant findings reported.
Resting EEG: theta power (4 to 7 Hz)	May be involved in biological rhythms and cognitive states. Increased awake theta power is related to neurological and psychiatric conditions.	Equivocal (both increase and decrease reported).	No abnormal theta power found.
Resting alpha power (8 to 12 Hz)	Higher cognitive function and brain maturation; integrative brain function.	Equivocal (both increase and decrease reported).	Equivocal (both increase and decrease reported).
Resting EEG: beta power (12 to 28 Hz)	Indicative of awake and active state. Increased beta may be related to increased neural excitability.	Increased power.	Increased power.
Resting EEG: dipole source modeling	Brain sources of scalp potentials. Abnormal source activity may be seen in clinical conditions.	No studies as yet.	No studies as yet.
Resting EEG: coherence	Functional connectivity between brain regions. Frequency-specific and region-specific coherence indicative of strength of coupling, network interaction, and brain maturation.	Increased high theta coherence; inconclusive in other frequencies.	Tenuous findings of increased coherence in several frequency bands.
EEG/event-related oscillations (ERO): graph theoretical method	Topological properties (i.e., regions and connectivity) of brain networks.	Graph theoretical indices of EEG data specific to alcoholic subjects have been elicited.	No studies as yet.
Resting EEG: microstate analysis	Possible indices of resting state networks in the brain.	No studies as yet.	No studies as yet.
EEG trilinear modeling	Estimation of a set of spatial and spectral components of brain potentials.	Significant linkage and association between trilinear component of EEG. beta band and a gamma-aminobutyric acid type A ($GABA_A$) receptor gene (*GABRA2*) in Collaborative Study on the Genetics of Alcoholism (COGA) densely affected alcoholic families.	No studies as yet.
EP: auditory brainstem potentials	Integrity of sensory pathways; sensory processing.	Prolonged latencies in several auditory brainstem potential peaks.	No change in amplitude or latency.
EP: P1/P100	Basic perceptual processing of the stimulus; modulated by physical characteristics of the stimulus.	Decreased amplitudes, delayed latencies and topographic changes in visual paradigms.	No significant findings reported.
Event-related potential (ERP): N1/N100	Attentional modulation during perceptual processing of the stimulus; selective attention.	Decreased amplitude.	Decreased amplitude.
ERP: MMN	Automatic stimulus change detection; central auditory processing mechanism.	Findings are equivocal.	Findings are equivocal.
ERP: ERN/Ne	Preconscious error-detection mechanism.	Findings are equivocal.	No studies as yet.
ERP: N2/N200	Detection of response conflict (conflict monitoring); response inhibition; feedback processing.	Decreased amplitude and delayed latency.	Decreased amplitude and delayed latency.

Table Summary of Major Electrophysiological Findings in Alcoholism *continued*

Method/Measure	Function/Dysfunction	Findings in Alcoholics	Findings in High-Risk (HR) Offspring/Relatives
ERP; P3/P300	Context/demand processing; stimulus significance; conscious attention; working memory.	Decreased amplitude and delayed latency.	Decreased amplitude and delayed latency.
ERP: N4/N400	Language/semantic processing; detection of incongruity in word meaning; semantic priming effects.	Decreased amplitude and delayed latency in word incongruity studies; lack of attenuation to primed words and no differentiation between primed vs. unprimed words (no priming effect).	Lack of attenuation to primed words; no differentiation between primed vs. unprimed words (no priming effect).
ERP: dipole source modeling	Brain sources of scalp potentials. Abnormal source activity may be seen in clinical conditions.	Changes in the location of brain sources for P1, N1, P2, and MMN.	No studies as yet.
ERP: current source density (CSD)	Estimation of the local radial current density and flow; spatial filtering; identification of neural sources. Changes in source activity in strength or location may suggest abnormality.	Changes in the topography and strength of activation for P3.	Changes in the topography and strength of activation for P3.
ERP: low-resolution brain electromagnetic tomography (LORETA)	Estimation of current density in voxels; identification of neural sources; patterns of activation and connectivity. Changes in current density activation level and pattern may suggest abnormality.	Changes in current density activation level and pattern for N2 and P3.	Changes in current density activation level and pattern for N2 and P3.
ERP: principal component analysis (PCA)	Decomposition of signals into orthogonal components representing distinct topographic activity patterns.	No conclusive findings.	No conclusive findings.
ERP: independent component analysis (ICA)	Decomposition of signals into a sum of temporally independent and spatially fixed components.	Changes in activation strength in ICA components for N2 and P3.	No studies as yet.
ERP: trilinear modeling	Estimation of a set of spatial and temporal components of brain potentials; simultaneous comparison of components across subjects and conditions is possible.	Significant linkage between time warped P3-related trilinear components in visual oddball paradigm in COGA densely affected alcoholic families.	No studies as yet.
ERO: delta (1 to 3.5 Hz) power	Signal detection and decision making; context/reward processing.	Decreased evoked and total delta power during P3 response window.	Decreased evoked and total delta power during P3 response window.
ERO: theta (3.5 to 7.5 Hz) power	Conscious awareness; episodic retrieval; recognition memory; executive control; inhibitory processing; working memory.	Decreased evoked and total theta power during N2 and P3 time window.	Decreased evoked and total theta power during N2 and P3 time window.
ERO: gamma (29 to 45 Hz) power	Visual perception, cognitive integrative function such as "binding", and top-down (frontal) control during sensory processing.	Reduction in early evoked gamma power at frontal regions during target processing.	Reduction in early evoked gamma power at posterior regions during target processing.
ERO: event-related desynchronization and synchronization (ERD/ERS)	ERD represents an activated cortical area with increased excitability, while ERS indicates a deactivated cortical area with decreased excitability.	No studies as yet.	No studies as yet.

Table Summary of Major Electrophysiological Findings in Alcoholism *continued*

Method/Measure	Function/Dysfunction	Findings in Alcoholics	Findings in High-Risk (HR) Offspring/Relatives
ERO: coherence	Functional interaction and connectivity across brain regions.	Increased wavelet coherence in theta (4 to 8 Hz), alpha (8 to 13 Hz) and gamma (50 to 60 Hz) bands at frontal and occipital regions during 100 to 200 ms poststimulus of target processing.	No studies as yet.
ERO: phase synchronization	Functional interactions and connectivity across brain regions; long-range neural integration.	Impaired synchronization and loss of lateralization, most prominently in alpha and lower beta frequency bands during mental rehearsal of pictures.	No studies as yet.
EEG/ERO: Granger causality	Directional influences and pathways in neural networks; couplings (connectivity) and information exchange across brain regions.	No studies as yet.	No studies as yet.

nology enhances the opportunity for further applications in clinical research in all spheres, many more tools are being developed in the electrophysiological arsenal to be effectively used to address the current and future challenges.

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Utilization of Magnetic Resonance Imaging in Research Involving Animal Models of Fetal Alcohol Spectrum Disorders

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It is well recognized that fetal alcohol exposure can profoundly damage the developing brain. The term fetal alcohol spectrum disorder (FASD) describes the range of deficits that result from prenatal alcohol exposure. Over the past two decades, researchers have used magnetic resonance imaging (MRI) as a noninvasive technique to characterize anatomical, physiological, and metabolic changes in the human brain that are part of FASD. As using animal models can circumvent many of the complications inherent to human studies, researchers have established and explored a number of models involving a range of species. Using MRI-based modalities, the FASD animal models have demonstrated decreased brain volume and abnormal brain shape, disrupted cellular morphology differentiation, altered neurochemistry, and blood perfusion. These animal studies have facilitated characterization of the direct effects of ethanol; in many cases identifying specific sequelae related to the timing and dose of exposure. Further, as a result of the ability to perform traditional (such as histological) analyses on animal brains following neuroimaging experiments, this work leads to improvements in the accuracy of our interpretations of neuroimaging findings in human studies.

Key words: Fetal alcohol exposure; prenatal alcohol exposure; fetal alcohol spectrum disorder; fetal alcohol effects; brain; developing brain; fetal development; central nervous system; magnetic resonance imaging; neuroimaging; animal models

Neuroimaging, particularly magnetic resonance imaging (MRI), has begun to tease apart the underlying mechanisms behind alcohol's deleterious effects on the fetus and eventually may lead to earlier detection of what can be devastating child neurodevelopmental deficits. In 1968, researchers first reported an association between prenatal alcohol exposure and what can be persistent adverse cognitive, behavioral, motor, and psychosocial outcomes, leading to the first description of fetal alcohol syndrome (FAS) (Jones and Smith 1973). FAS, as described by prenatal and/or postnatal growth retardation, central nervous system (CNS) involvement, and facial dysmorphology, represents some of the most extreme effects of maternal alcohol use. However, there is a broader spectrum of symptoms, with some individuals prenatally exposed to alcohol having significant neurobehavioral deficits but not the full FAS symptomology (Mattson et al. 1997). To better represent the effect of alcohol on children prenatally exposed to alcohol, clinicians and researchers now use the term fetal alcohol spectrum disorder (FASD) (Mattson et al. 1998).

Although researchers have established a causal relationship between fetal alcohol exposure and life-long cognitive and behavioral impairment, it remains less clear how changes in the developing brain mediate these impairments. In addition, the detection of FASD remains elusive as the diagnostic criteria of FAS/FASD typically only allow for identification of affected individuals in late childhood. Noninvasive neuroimaging techniques hold potential for both identifying the underlying mechanisms behind alcohol's deleterious effects on the central nervous system (CNS) and helping detect FAS/FASD much earlier. And although studies in humans have provided some insight into these issues, studies in animals allow researchers to ask far more detailed questions.

MRI Techniques in Humans

MRI is a safe, noninvasive neuroimaging method that allows repetitive examination of human brains. It provides relatively high spatial resolution (approximately 1 × 1 × 1 mm^3 in most modalities) and a rich toolbox that enables researchers to perform anatomical, physiological, and metabolic measurements (see sidebar on "Magnetic Resonance Imaging Techniques" for detailed descriptions of the various techniques). Over the past two decades, various MRI

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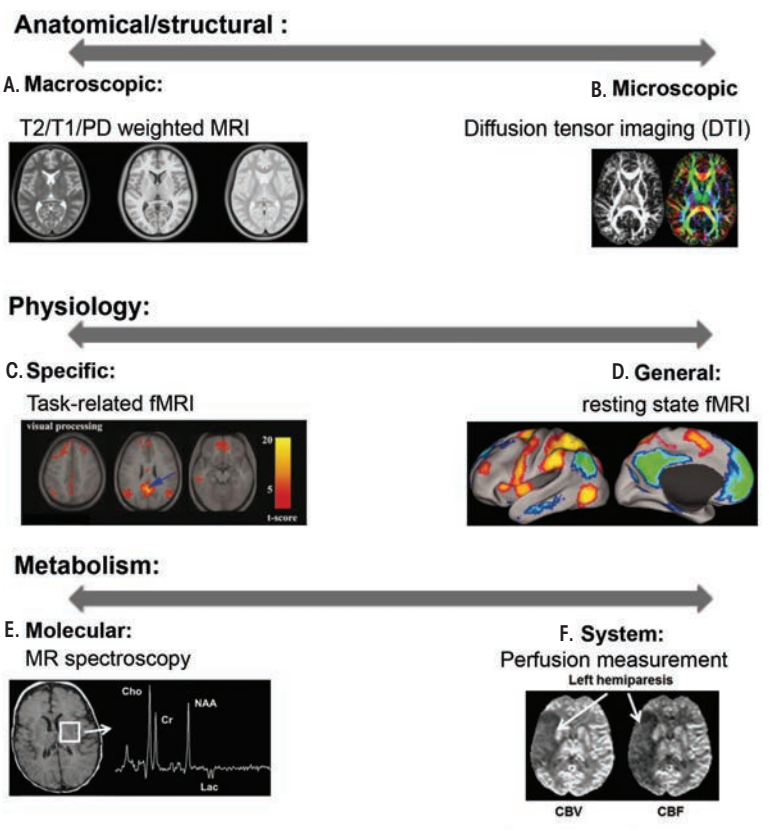
Magnetic Resonance Imaging [MRI] Techniques

To produce image contrast, conventional MRI utilizes the fact that water 1H nuclei of different tissue types have different T1 and T2 relaxation times. By varying data acquisition parameters such as time of repetition (TR) and/or time of echo (TE), contrast can be tuned to enhance differentially anatomical structures such as gray matter, white matter, and cerebrospinal fluid (CSF). As a result, imaging can segment specific anatomical structures such as basil ganglia, cerebellum, corpus callosum, and hippocampus to facilitate quantitative volume and shape analyses (for the definition of these and other terms, see Glossary).



SOURCES: C. Greicius et al. 2003; D. Fox et al. 2005; E. Gujar et al. 2005; F. Calamante et al. 1999

Diffusion Tensor Imaging (DTI)

Water diffuses through biological tissues based on thermally driven Brownian motion and is impeded by myriad structures. During the typical diffusion time in a diffusion magnetic resonance (MR) scan (10 to 100 ms), the behavior of water diffusion within the central nervous system (CNS) can vary dramatically depending on the tissue subtype. In cerebrospinal fluid, water experiences free and isotropic diffusion, which means it moves equally in all directions. In mature white matter and some gray matter regions (e.g. hippocampus, cerebellum, and cerebral cortex), interactions with biological membranes significantly reduces water diffusion perpendicular to dominant cellular processes (axons, dendrites, and glial processes). Thus, the diffusion is direction dependent, or what is known as anisotropic. In gray matter regions that lack highly oriented cellular structures, water molecules experience boundaries in a more random fashion and this situation often is referred to as restricted isotropic diffusion.

For each imaging voxel, DTI measurements can derive multiple parameters. One commonly used parameter is fractional anisotropy (FA), which characterizes the degree of anisotropy of a diffusion process. FA measurements range between 0, which represents isotropic diffusion as in free water or cerebrospinal fluid (CSF), and 1, which indicates that diffusion is completely restricted along one or more directions. Therefore, high FA reflects coherent and highly orientated fiber tracts and decreased FA often indicates myelin and axon injury, and/or any disruption of fiber tracts. Mean diffusivity (MD) is a scalar measure of the total diffusion within a voxel and reflects the mobility of water molecules. MD is generally high in CSF, and lower in normal gray and white matter. Compared with anatomical MRI, DTI-derived metrics are more sensitive to the changes on a cellular level (Mori and Zhang 2006).

Functional MRI (fMRI)

fMRI is a technique that measures brain physiological activity. It does so based on the coupling of blood flow and neuron activity and the difference in water 1H spin relaxation between environments of deoxyhemoglobin and oxyhemoglobin.

Magnetic Resonance Imaging [MRI] Techniques *continued*

In typical task-based fMRI experiments (Logothetis 2008), a subject alternates between a specific task-responding state and a control state. In brain areas where a task activates neurons, blood flow is altered such that more oxyhemoglobin is present compared to deoxyhemoglobin. This results in a transient task-dependent increase in magnetic resonance (MR) signal intensity within the brain regions that the task activates and this phenomenon is termed blood oxygen level dependent (BOLD) MR signal.

In resting-state fMRI experiments (Fox et al. 2005), no stimulus is presented to the subject, and temporally correlated MR intensity fluctuations are used to infer disparate brain areas that are functionally related to each other.

MR Spectroscopy (MRS)

MR spectroscopy provides a quantitative and specific measure of brain chemistry. While conventional MRI primarily detects water 1H nuclei, MRS detects proton signals from other molecules such as amino acids (e.g. glutamate), lipids, lactate, N-acetylaspartate (NAA), choline, creatine, and, when present, ethanol. Alternatively, MRS can also detect other MR active nuclei (e.g. 31P, 23Na, 19F, etc). Researchers can use MRI and MRS in combination: MRI to identify an anatomical location and localized MRS to detect the concentration of specific metabolites within the region of interest. Among the MRS studies of human and animal models of FASD, researchers most frequently examine NAA, choline-containing compounds (Cho), and creatine/phosphocreatine (Cr) signals. The NAA signal includes contributions from primarily NAA, and to a lesser extent from N-acetylaspartylglutamate (NAAG). NAA is considered to be a marker that reflects neuronal/axonal health, viability and density. Cho signals consist of multiple choline derivatives which are precursors or degradation products of the membrane phospholipids. Thus, the Cho signal is seen as a marker for cell membrane integrity and myelination. The Cr signal, constituted of both creatine and phosphocreatine, is thought to reflect energy phosphate metabolism. As the absolute measurements of signal intensities of these metabolites are subject to source errors including CSF contamination, the Cr peak, which is relatively constant between individuals and most brain areas, is often used as internal reference. Thus in this review, we only discuss the NAA/Cr and Cho/Cr ratios (Graaf 2002).

MR Perfusion Measurements

Cerebral blood flow (CBF), is the blood supply to the brain at any given time and is tightly regulated to meet the brain's metabolic demands. In an adult, CBF is typically 750 ml/min. This equates to an average perfusion of 50–54 ml of blood per 100 g of brain tissue per minute. A number of MR modalities can be used to measure blood perfusion within the brain, such as dynamic susceptibility contrast (DSC) MRI, dynamic contrast enhanced (DCE) MRI, and arterial spin labeling (ASL).

DSC-MRI involves the injection of a bolus paramagnetic contrast agent. Then a fast imaging sequence is used to acquire a series of T2*-weighted images during the contrast agent's first passage through the tissue. The passage of the contrast agent leads to MR signal intensity drop due to the magnetic susceptibility effect. The signal intensity-time curve measured by the series of T2*-weighted images can be mathematically converted to a contrast agent concentration-time curve. The concentration-time curve is then integrated to give an index that is proportional to the relative cerebral blood volume (rCBV) of a given imaging voxel. Additionally, if such measurement is done within or near a major artery, the arterial input function can then be derived and in turn, relative cerebral blood flow can also be calculated (Calamante 1999).

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techniques have uncovered brain abnormalities that are associated with cognitive/behavioral deficits in FASD-affected individuals (Riley et al. 1995, 2004).

Anatomical Differences

Traditional MRI studies show anatomical differences between the brains of children and adolescents with FASD and those not exposed to alcohol in utero, including the following:

- Significant reductions in overall brain volumes in children and adolescents with FASD (Archibald et al. 2001; Johnson et al. 1996; Lebel et al. 2008; Sowell et al. 2002; Willoughby et al. 2008);
- Reduced volumes in specific regions, including the caudate nucleus (Archibald et al. 2001; Cortese et al. 2006), hippocampus (Willoughby et al. 2008), and cerebellar vermis (Autti-Ramo et al. 2002); and
- Corpus callosum malformations in FASD individuals (Autti-Ramo et al. 2002; Bookstein et al. 2002; Johnson et al. 1996).

Study results are mixed regarding the effect of maternal ethanol exposure on fetal cerebral cortical thickness: Sowell and colleagues (2002, 2008) observed greater cortical thickness in parietal and posterior temporal regions, whereas Zhou and colleagues (2011) reported thinner cortical gray matter in a number of brain regions in an FASD group. Meanwhile, results from studies using diffusion tensor imaging (DTI), which allows researchers to assess tissue abnormalities on a microstructural level, even in the absence of gross dysmorphology, suggest that individuals exposed to alcohol in utero have less organized white matter fiber tracts (Wozniak and Muetzel 2011). Specifically, DTI showed significantly decreased fractional anisotropy and/or increased mean diffusivity (MD) in the corpus callosum (Ma et al. 2005) and other white matter regions (Fryer et al. 2009; Lebel et al. 2008; Sowell et al. 2008) of alcohol-exposed individual compared with those who were not exposed.

Neurochemical Changes

Another technique, magnetic resonance spectroscopy (MRS) (see sidebar on "Magnetic Resonance Imaging Techniques"), offers a unique way to detect neurochemical changes by monitoring the concentration of neurometabolites, including choline- containing compounds (Cho), which are markers of cell membrane stability and myelination; N-acetylaspartate (NAA), a marker of neuronal/axonal viability and or density; and creatine/phosphocreatine (Cr), a marker of metabolic activity (Moffett et al. 2007). One MRS study (Fagerlund et al. 2006) comparing people with FAS with normal control subjects reported lower NAA levels in various brain regions among the FAS subjects, whereas another study (Cortese et al. 2006) found higher NAA levels in the caudate nucleus.

Brain Activation Patterns

Functional MRI allows researchers to detect differences in brain activation patterns between FASD individuals and control subjects during various tasks involving spatial, verbal, and visual working memory (Astley et al. 2009; Malisza et al. 2005; O'Hare et al. 2009), verbal learning (Sowell et al. 2007), and inhibitory control (Fryer et al. 2007). These altered brain activation patterns might underlie the poor executive functioning-based skills observed in FASD individuals (Astley et al. 2009; Fryer et al. 2007; Malisza et al. 2005; O'Hare et al. 2009; Sowell et al. 2007).

Drawbacks of Human Studies

Although neuroimaging and neuropathological investigations of the brains of FASD-affected individuals have elucidated specific abnormalities in brain structure, metabolism, and function underlying cognitive and behavioral impairments, studies of human subjects have a number of limitations. These include (1) the paucity of autopsy reports from children with FASD hampers interpretation of in vivo human neuroimaging data; (2) the related inability to evaluate and validate correlative structural and functional damage; (3) the difficulty in controlling, or even determining, variables such as dosing, timing, and consumption pattern of maternal drinking; and (4) the difficulty in eliminating confounds in human studies including environment, other maternal substance abuse, stress, and malnutrition. Animal models of FAS/FASD circumvent many of these inherent complications.

MRI in Animals

Animal models allow researchers to control maternal and environmental variables such as genetic background, nutritional status, dosage, and timing pattern of ethanol insult, which frequently enables experiments to focus on the mechanisms of ethanol's teratogenic action. As early as 1977, studies of mouse and rat FASD models confirmed the causal relationship between prenatal alcohol exposure and FASD, which had been speculated in clinical observations (Abel and Dintcheff 1978; Chernoff 1977). Shortly after, Sulik and colleagues (1981) demonstrated that treating pregnant mice with alcohol at gestation day (GD) 7 (equivalent to human gestation week [GW] 3) resulted in facial dysmorphology in their offspring, a finding consistent with FASD-affected human infants. Since then, researchers have established a number of animal models in a range of species to study the mechanism of alcohol's teratogenic effects, to test the efficacy of protective interventions, and to improve the sensitivity and specificity of neuroimaging techniques for identifying FASD (see figure 1). Each model provides certain advantages and disadvantages as described below.

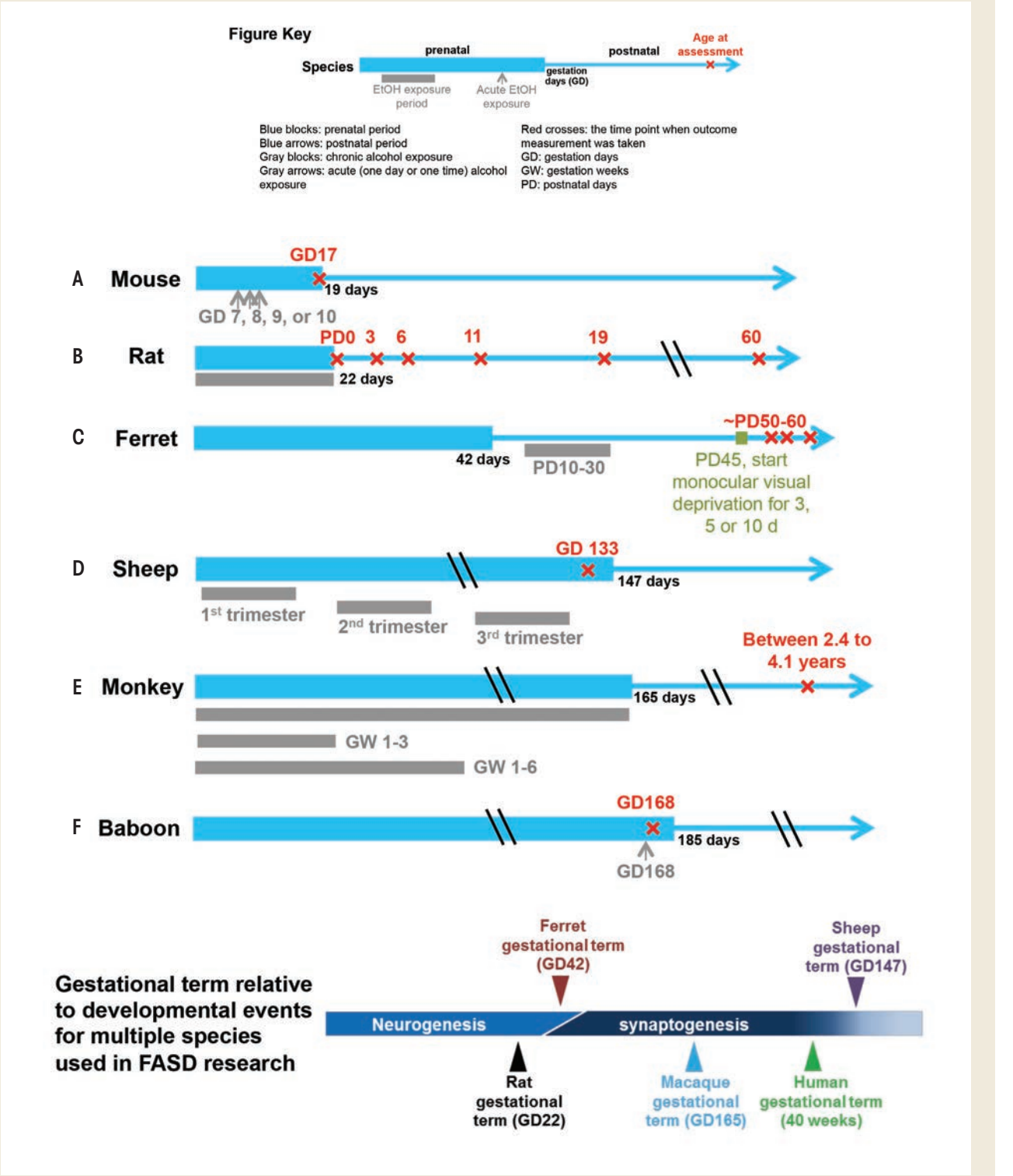

Figure 1 Timing schemes of popular animal models for FASD research.

Nonmammalian Animals

Nonmammalian animal models, including zebra fish, fruit fly, and frog, offer unique advantages by providing flexible and well-characterized experimental systems. However, more phylogenetically advanced vertebrate species are necessary in studies that require a complex CNS and long developmental periods.

Rodents

Among mammalian species with a more complex CNS, rodents are a very important model system. They have a short reproductive cycle, their genetic background is readily controlled, and their small size makes them suitable for smallbore animal MRI systems.

Ferrets

Ferrets also have a fairly short pregnancy period relative to CNS developmental milestones (see figure 1C) and, as a result, are advantageous for investigating early neurodevelopmental disruption without the complication of needing to induce premature delivery, or perform in utero manipulations.

Sheep

Sheep have a long gestational term relative to CNS development, more resembling human gestation, which allows investigators to explore various drinking patterns and exposure times more similar to those seen in humans.

Nonhuman Primates

The CNS develops very similarly in nonhuman primates and humans. In addition to their large and highly folded brains, nonhuman primates also exhibit more complex social relationships and cognitive functions. Thus, they can serve as a bridge between studies in other animal models and humans (Miranda-Dominguez et al. 2014). In a number of cases, studies conducted on FASD animal models have productively used MRI as a noninvasive neuroimaging modality. The primary outcomes from studies in all of these models, which are reviewed below, are summarized in the table.

Findings From Animal Models

Rodents

In a series of mouse FASD studies, researchers used ex vivo MRI to examine the effect of acute ethanol insult on GD 7, 8, 9, and 10, a time range that corresponds to human GWs 3 to 4 (Godin et al. 2010; O'Leary-Moore et al. 2010; Parnell et al. 2009, 2013). To characterize ethanol-induced structural brain abnormalities, they analyzed high-resolution MR images of each fetus dissected on GD 17 (see figure 2). They measured key growth metrics such as brain width, mid-sagittal brain length, and third ventricle width in a single image plane (see figure 2A). They also segmented and then reconstructed regional brain structures (e.g. cerebral cortex, ventricles, cerebellum, etc.) to quantify their volume and morphology in three dimension (see figure 2B and C). In the fetuses exposed to ethanol in utero, the researchers found notable volume reductions across various brain regions, which were accompanied by increased ventricular sizes. They also observed regional brain morphology changes including holoprosencephaly, or the absence of midline cerebral structures, and widened space between cerebral hemispheres (see figure 3B and C). These results demonstrate that an acute maternal alcohol insult on GD 7 to 10 leads to a spectrum of forebrain deficiencies in mouse fetuses. Importantly, some animals that exhibited CNS malformations did not have facial dysmorphology. This series of studies employing an acute, high-dose maternal ethanol treatment paradigm helped titrate sensitive periods for a variety of malformations and extended our knowledge of the dependency of ethanol teratogenesis on the timing of exposure during gestation.

A more recent study conducted by the same research group examined brain dysmorphology resulting from maternal dietary ethanol intake at a much lower dose than the previous study and occurring during the time period equivalent to the first trimester in humans (Parnell et al. 2014). Using the same MRI-based volumetric measurements, the researchers observed reduced cerebellum and enlarged septal region in a GD 7 to 11 ethanol-exposure group. In a GD 12 to 16 ethanol-exposure group, the researchers detected size reductions in right hippocampus and increased pituitary gland volume. Overall, the number of brain regions significantly affected and the severity of the effect were less than those following acute, high-dose exposures. The application of high-resolution MRI here has facilitated the systematic and comprehensive examination of the brain abnormalities caused by prenatal ethanol exposure. The employment of a mouse FASD model in these studies allowed the control of variables, especially ethanol exposure patterns, which, in turn, aided in confirming that the type and severity of ethanol-induced birth defects largely depend on the treatment pattern and dosage along with the developmental stage at the time of ethanol exposure (Godin et al. 2010; O'Leary-Moore et al. 2010; Parnell et al. 2009, 2013, 2014).

A study in rats using ex vivo high-resolution MRS examined regional neurochemistry in frontal cortex, striatum, hippocampus, and cerebellum in postnatal day [PD] 16 animals exposed to ethanol as neonates (PD 4 to 9) (O'Leary-Moore et al. 2008). The technique allowed them to measure the relative concentrations of certain brain metabolites, comparing the brains of ethanol exposed rats with those of control rats. They found changes in several metabolites in various brain regions:

- The NAA/Cr ratio was reduced in the cerebellum, which likely reflects delayed development, cell loss, or both in these regions. This finding supports those of a human FASD study (Fagerlund et al. 2006), reporting a decrease in NAA/Cr in cerebellum along with other brain regions. That said, another human FASD study (Cortese et al. 2006) reported an increased NAA/Cr ratio in the caudate nucleus in FASD individuals. The researchers suggested that this increase might be indicative of a "lack of normal programmed cell death, dendritic pruning/myelination during development" (p. 597).
- The Cho/Cr ratio was significantly lowered in hippocampus and elevated in striatum in ethanol-exposed rats

Table Findings of Magnetic Resonance (MR)-Based Fetal Alcohol Spectrum Disorder (FASD) Animal Studies.

MR Modalities	MR Studies	Ethanol Exposure	Age at Assessment	Findings
Anatomical MRI	Astley et al. 1995 Monkey	Weekly, gestation week (GW) 1 to 3, or 1 to 6, or 1 to 24, 2 g/kg, intragastric gavage	2 to 4 years	No gross morphological abnormalities. No gross difference in size of cerebral hemispheres, corpus callosum, brain stem, or cerebellum.
	Godin et al. 2010 Mouse	Gestation day (GD) 7, 2.9 g/kg, intraperitoneal (i.p.) injection	GD 17	Holoprosencephacy (the forebrain fails to develop), cerebral cortical heterotopia (where clumps of gray matter develop in the wrong places), failure of the pituitary gland to develop (pituitary agenesis), dilation of the third ventricle.
	Parnell et al. 2009 Mouse	GD 8, 2.9 g/kg, i.p. injection	GD 17	Reduction of total brain volume. Comparison of individual regions revealed difference in all except the pituitary and septum.
	Parnell et al. 2013 Mouse	GD 9, 2.9 g/kg, i.p. injection	GD 17	Increase in septal region width, reduction in cerebellar volume, ventricular dilation, malformation of cerebral cortex, hippocampus and right striatum.
	O'Leary-Moore et al. 2010 Mouse	GD 10, 2.9 g/kg, i.p. injection	GD 17	Ventricular dilation, reduction in total brain volume as well as each of the assessed brain structures.
	Parnell et al. 2014	GD 7 to11, 4.8 percent EtOH-containing diet (vol/vol)	GD 17	Decrease in cerebellar volume, increase in septal volume.
	Mouse	GD 12 to 16, 4.8 percent EtOH-containing diet (vol/vol)	GD 17	Reduction of right hippocampal volume, increase in pituitary volume.
	Leigland et al. 2013*a* Rat	Daily, GD 1 to 20, 4.5 g/kg, intragastric gavage	PD 0, 3, 6, 11, 19, 60	Reduction of brain and isocortical volumes, reduction of isocortical surface area and thickness.
Diffusion Tensor Imaging (DTI)	Leigland et al. 2013*b* Rat	Daily, GD 1 to 20, 4.5 g/kg, intragastric gavage	PD 0, 3, 6	Higher fraction anisotropy (FA) in cerebral cortex.
Magnetic Resonance Spectroscopy (MRS)	Astley et al. 1995 Monkey	Weekly, GW 1 to 3, or 1 to 6, or 1 to 24, 2 g/kg, intragastric gavage	2 to 4 years	Increased Cho/Cr with increased duration of EtOH intake.
	O'Leary-Moore et al. 2008 Rat	Daily, postnatal day (PD) 4 to 9, 5 g/kg intragastric gavage	PD 16	Increased NAA/Cr in cerebellum and striatum, Cho/Cr ratio was increased in striatum but decreased in hippocampus.
Perfusion MRI	Kochunov et al. 2010 Baboon	GW 24, 3 g/kg, intragastric gavage	Immediately following ethanol exposure	Increased permeability of placental membrane, increased cerebral blood flow in fetal brain.

compared with controls. The researchers concluded that these changes in Cho level were "consistent with dysfunctional membrane turnover in the young perinatal ethanol-exposed brain" (p. 1704).

- The concentration of the amino acid taurine was reduced in hippocampus and striatum. Taurine deficits can cause growth retardation and impaired CNS function (Aerts and Van Assche 2002).
- Glutamate, an excitatory neurotransmitter, was reduced in cerebellum only from prenatal ethanol-exposed female rats, indicating disrupted glutamatergic function (O'Leary-Moore et al. 2008).
- A trend of decreased γ-aminobutyric acid (GABA) (without statistical significance) also was observed in the striatum and cerebellum in the rats with neonatal ethanol exposure.

The ability to study this broad range of MRS signals in animal models may hold potential for the development of additional biomarkers for FASD diagnosis and treatment evaluation.

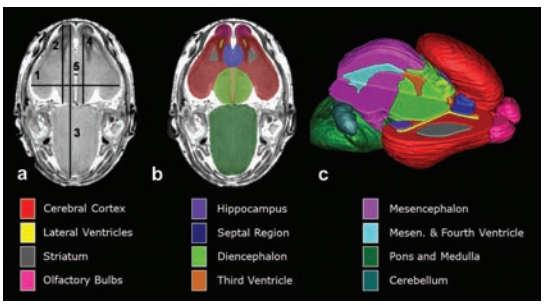

Figure 2 High-resolution magnetic resonance (MR) images of mouse fetuses at gestational day (GD) 17 allow for linear measurements, regional segmentation, and three-dimensional reconstruction. **(A)** A horizontal image with lines depicting sites of linear measurement as follows: brain width (biparietal distance), line 1; bulbothalamic distance, line 2; mid-sagittal brain length, line 3; frontothalamic distance, line 4; third ventricle width, line 5. (Cerebellar width [transverse cerebellar distance, not included] was measured at its greatest dimension.) Manual segmentation, as depicted by the color-coded regions in **(B)** allowed for subsequent three-dimensional reconstruction **(C)** and analyses of selected brain regions. **(C)** The upper right quadrant of the brain has been removed to allow for visualization of the interior structures. Color codes for the segmented brain regions shown are at the bottom of the figure.

NOTE: Figure adapted from (Godin et al. 2010).

In another rat study (Leigland et al. 2013*a*), researchers used ex vivo MRI to examine the cerebral cortex of rat pups born to dams treated with ethanol throughout gestation, comparing them with pups whose moms either received maltose/dextrin instead of ethanol or no treatment. They performed cross-sectional measurements on the pups on PD 0, 3, 6, 11, 19, and 60 (see figure 1B). The ethanol-exposed pups had reductions in volume, thickness, and surface area of the cerebral cortex on PD 0, compared with control and M/D-treated groups, and the difference persisted into adulthood (PD 60). To examine whether prenatal ethanol exposure differently affected particular areas of the cerebral cortex, the researchers analyzed differences in regional patterns of cortical thickness. They saw a significant difference in the parietal and frontal-parietal region of the cortex or somatosensory and motor locations (see figure 4). This finding agrees with a human study observing smaller cortical thickness in FASD (Zhou et al. 2011) but is at odds with reports by Sowell and colleagues (2002, 2008, 2001), in which greater cortical thickness was reported in FASD-affected individuals. The discrepancy between the two human studies might be explained by differences in the image processing procedures used. Leigland and colleagues (2013*a*), in the rat study, and Zhou and colleagues (2011), in the human study, recorded absolute cerebral cortical thickness/volume; Sowell and colleagues (2001) normalized individual gray matter volume

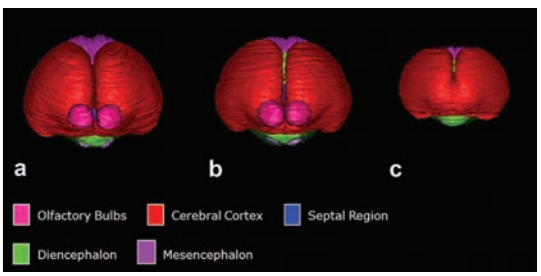

Figure 3 Reconstructed brains of a control fetal mouse at gestational age 17 **(A)** along with the brains of ethanol-exposed fetuses having mid-facial abnormality **(B** and **C)**. Segmented magnetic resonance microscopy scans of control **(A)** and ethanol-exposed **(B** and **C)** fetuses were reconstructed to yield whole brain (frontal view). Although the affected fetus in **(B)** had a normal-appearing face (figure not shown here), a slight widening of the space between the cerebral hemispheres (as evidenced by visibility of the septal region and diencephalon) can be seen as compared with control **(A)**. Missing olfactory bulb and rostral union of the cerebral hemispheres can be seen in fetus **(C)**.

NOTE: Figure adapted from (Godin et al. 2010).

to total brain volume before statistical analyses in their study. If fetal ethanol exposure disproportionately affects shrinkage of different brain structures, it is possible that differences in the direction of effect on cerebral cortical thickness could result from the different data processing strategies.

Although a majority of neuroimaging research on early cerebral cortical development has focused on gross volume change and dysmorphology, one study used ex vivo DTI on rats to characterize prenatal ethanol exposure's effect on cortical neuron morphological differentiation (Leigland et al. 2013*b*). Rats exposed to daily ethanol throughout gestation exhibited a higher diffusion fractional anisotropy (FA) in their cerebral cortex compared with age-matched M/D controls at ages PD 0, PD 3, and PD 6, indicating a higher preference for water to diffuse radially rather than parallel to the pial surface (figure 5) (see sidebar "Magnetic Resonance Imaging Techniques" for explanation of the technique). The researchers validated this finding with quantitative histological analyses of the same brains. They found that higher FA reflected a more simple and coherent cortical cellular structure, which has previously been shown with traditional invasive anatomical measurement methods (Cui et al. 2010; Davies and Smith 1981; Fabregues et al. 1985; Hammer and Scheibel 1981) to result from ethanol-induced disruption in neuronal differentiation. The framework proposed in this study in which cellular-level microstructure can be inferred by DTI-derived FA provides a novel strategy for characterizing the effects of ethanol exposure on cerebral cortical gray matter.

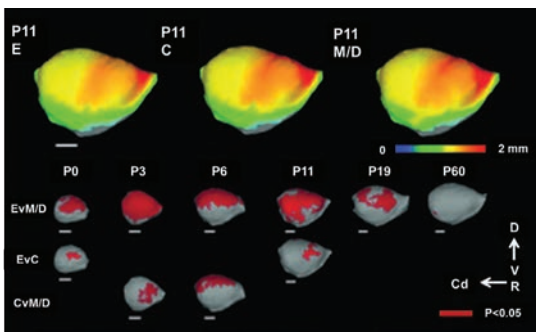

Figure 4 Regional pattern of cerebral cortical thickness differences result from threshold-free cluster enhancement (TFCE) analysis. On the top row, mean cortical thickness at postnatal day (PD) 11 for each group in the rat (n = 4 to 6/age/group) are projected onto target cortical surfaces. TFCE results are pictured in dark red in the last three rows representing regions in which mean cortical thickness between groups is significantly different ($P < 0.05$). Specific regional differences, centered on primary sensory areas were found among ethanol **(E)** and maltose/dextrin (M/D) groups at all ages. Regions of significant difference also were found in comparisons between E and control **(C)** groups at PD 0 and PD 11 and between control **(C)** and M/D groups at P 3 and P 6. Scale bars (in white) represent 2 mm. D, dorsal; V, ventral; Cd, caudal; R, rostral.

NOTE: Figure adapted from Leigland et al. 2013*a*.

Sheep and Ferret

Although, to our knowledge, no MRI studies have been published on fetal alcohol exposed sheep or ferrets, we review here some results using these species for FASD research. Gestational term lengths in these species, relative to other developmental events, represent extremes, and these properties have been exploited to address specific scientific questions. Similar to humans, sheep have a long gestation time and all three trimester equivalents occur in utero. Studies have found that binge ethanol exposure in all three trimesters leads to deficits in fetal cerebellar Purkinje cells (Ramadoss et al. 2007*a,b*) (see figure 1D). Another study using a sheep FASD model reported that second-trimester alcohol exposure has an adverse effect on fetal cerebral blood flow (Mayock et al. 2007). In contrast to sheep, ferrets have a short gestation time relative to CNS development, and its third-trimester equivalent of human gestation occurs postnatally. During this time, exposure to ethanol can disrupt neuronal differentiation, synaptogenesis, circuit formation, and remodeling of neuronal connections. Medina and colleagues (2003) have used a ferret monocular deprivation model, a well-characterized model of neuronal plasticity in the neocortex, to find that a 3-week alcohol exposure starting PD 10 impairs ocular dominance plasticity at a later age (see figure 1C), indicating ethanol insult during this time could have a profound effect on development and plasticity of neural circuits in the neocortex.

Nonhuman Primates

As early as 1995, Astley and colleagues used MRI and MRS to study brain structural and biochemical changes in a macaque monkey model of FASD (Astley et al. 1995). In this study, they explored three ethanol exposure patterns: once per week throughout the entire gestation period, once per week through GW 1 to 3, and once per week through GW 1 to 6 (see figure 1E). The researchers conducted MRI and MRS on the offspring of these treated monkeys between ages 2.4 and 4.1 years. Radiologists blinded to the monkeys' alcohol exposure inspected the MRI images and found no difference in morphology or size of cerebral hemispheres, corpus callosum, brain stem, or cerebellum. However, MRS from the thalamus, parts of the internal capsule, and basal ganglia detected a significant increase in Cho/Cr ratio with increasing duration of in utero ethanol exposure. Importantly, the study also found the Cho/Cr ratio to be associated with increased cognitive impairment as assessed by the Infant Development Impairment Score. Further analyses of NAA/Cr and NAA/Cho ratios suggested that the Cho component

changes with increasing ethanol exposure time. The researchers speculated that higher choline content might be associated with membrane breakdown.

A more recent study used dynamic susceptibility contrast (DSC)-MRI (see sidebar "Magnetic Resonance Imaging Techniques") to probe the effect of acute ethanol intake on pregnant baboons and their fetuses. Specifically, the study examined the effect of ethanol on the inner layer of the uterine wall, known as the myometrium, and on fetal brain perfusion, which is a measure of cerebral blood flow (CBF) (Kochunov et al. 2010). The researchers measured brain perfusion before (baseline) and immediately following the administration of an ethanol dose equivalent to human binge drinking (see figure 1F). In the fetal brain, the peak contrast uptake concentrations and contrast uptake and washout rates were significantly increased after ethanol treatment, suggesting that the ethanol increases CBF. The researchers hypothesized that ethanol's vasoactive properties are responsible for this CBF increase. This study also suggested that ethanol increased the permeability of placental membranes to the contrast agent, which is used to improve visibility of tissues during imaging. Specifically, the researchers found that more agent entered the fetal cerebral circulation, indicated by greater MR signal reduction in the fetal brain acutely following ethanol exposure. This is the first study to investigate ethanol's effect on fetal CBF and placenta permeability using in utero DSC-MRI. The study suggests two potential teratogenic mechanisms of ethanol: ethanol-mediated changes in placental permeability and ethanol-induced changes in fetal CBF.

Although MR studies of nonhuman primate FASD models are sparse, a number of studies using invasive methods have been conducted to examine alcohol's effect on the CNS in fetal ethanol exposed monkey fetuses. Multiple exposures of monkey fetuses to alcohol during specific developmental periods cause a reduced number of Purkinje cells in the cerebellum (Bonthius et al. 1996) and neurons in the frontal lobes (Burke et al. 2009). Two recent histological studies in fetal macaque monkeys found that an acute single exposure to alcohol during the third trimester causes widespread neuron apoptosis throughout gray matter regions (Farber et al. 2010) and glial cell (of the oligodentrocyte lineage) apoptosis across white matter regions (Creeley et al. 2013). These disruptions on a cellular level might contribute to the observed changes in neurometabolites observed in MRS studies in both human and animal FASD (Astley et al. 1995; Cortese et al. 2006; Fagerlund et al. 2006; O'Leary-Moore et al. 2008).

Summary and Future Directions

The use of animal models in FASD studies has deepened our understanding of the biological bases of FASD, improving the accuracy of our interpretations of neuroimaging findings in human studies, and provided potential markers for future FASD diagnosis. A significant current goal of many research groups is the development of new noninvasive strategies for early detection of deleterious effects of prenatal ethanol exposure. As Streissguth and colleagues (2004) have noted, the odds ratios of several adverse life outcomes decrease in FASD individuals when therapeutic intervention strategies are initiated early in life. The rationale for this observation has been twofold: CNS plasticity decreases over the first few years of life (Olson et al. 2007), and early diagnosis of FASD is particularly important as it allows "capable caring families to advocate for their children's needs (p. 235)" before establishment of maladaptive behavior (Streissguth et al. 2004). For these reasons, the design of methods for early detection of prenatal ethanol exposure-induced perturbation of normal development remains an important objective in applications of noninvasive neuroimaging tools and animal models of FASD.

As this overview has shown, many laboratories are engaged in research using animal models of FASD. They are implementing studies that vary the timing of ethanol exposure relative to CNS development, along with a diverse array of MRI modalities so they can better understand the consequences of ethanol exposure on anatomical, physio-

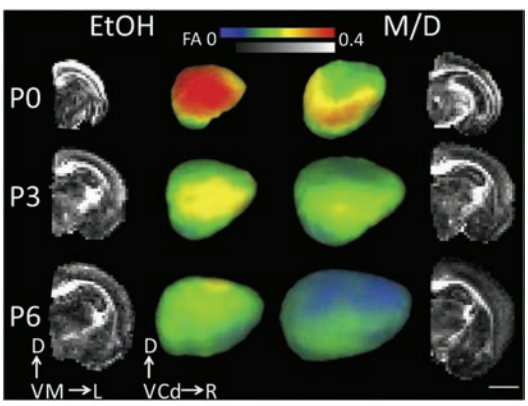

Figure 5 Effect of prenatal ethanol exposure on cerebral cortical fractional anisotropy. The two middle columns of images are laterally facing mid-cortical surface models of one rat at PD 0, PD 3, and PD 6 right hemisphere for each treatment group (ethanol) and maltose/dextrin (M/D), on which cortical fractional anisotropy (FA) at each mid-cortical surface node is projected. The outer columns represent mid-coronal FA maps for the right hemisphere of the same subjects depicted in the middle columns. Cortical FA decreased significantly with age. Additionally, cortical FA was largest, and isocortical volume smallest, in the ethanol group compared with the M/D group. This group difference is most visible in the outer layers of the cortex.

NOTE: Scale bar is 4 mm. D = dorsal, V = ventral, M = medial, L = lateral, Cd = caudal, R = rostral. Figure adapted from Leigland et al. 2013*b*.

logical, and metabolic development (see table). Among the advantages of using noninvasive neuroimaging techniques in animal models is the translatability of findings to clinical studies. In many cases, the biological bases of neuroimaging results obtained in human studies are not well understood. In these cases, parallel neuroimaging experiments with animal models can be performed, and interpretations of the findings can be validated with independent (but often invasive) experimental approaches. Efforts are being made to bridge the MRI findings to histopathological results, which are thought to be the gold standards in and outside FASD research (Jespersen et al. 2012; Leigland et al. 2013*b*; Riddle et al. 2011). In many other cases, studies using MR techniques to monitor CNS development and to examine CNS pathologies (other than FAS/FASD) also can provide valuable perspectives for FASD research. For example, ongoing diffusion MR microscopy efforts have been used to provide a detailed quantitative description of embryonic and early postnatal mouse brain development (Aggarwal et al. 2014; Zhang et al. 2003, 2006). Diffusion anisotropy maps derived from this method show excellent tissue contrast and, as a result, allow visualization of fine microstructural detail of the developing brain. This technique will be useful for investigation of ethanol-induced brain abnormalities in animal models over this age range. In addition, the development of advanced motion correction and imaging reconstruction technique has made in utero MRI examinations possible in humans as well as animals (Fogtmann et al. 2014). Using reconstructed in utero MRI, researchers can delineate human fetal brain tissues (including transient structures present only at early stages of development such as the cortical plate, intermediate zone, ventricular and subventricular zones, etc.) and can plot their growth trajectories (Scott et al. 2011). This close monitoring can help identify fetuses with growth patterns that deviate from the normal trajectory. In turn, their later cognitive outcome could be associated with growth patterns in future studies.

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The authors declare that they have no competing financial interests.

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Glossary

Apoptosis: A process of programmed cell death.

Basal ganglia: A part of the brain located at the base of the forebrain and strongly interconnected with several brain regions, including the cerebral cortex, thalamus, and brainstem. The basal ganglia are associated with a variety of functions, including control of voluntary motor movements, procedural learning, and routine behaviors.

Caudate nucleus: One of three basic structures in the brain that make up the basal ganglia and is part of a system that is largely responsible for voluntary movement.

Cerebellum: The part of the brain important for motor control and other functions.

Corpus callosum: A wide, flat bundle of neuronal fibers that connect the left and right hemispheres of the brain.

Ex vivo: Experiments or measurements done on cells or tissues removed from an organism. Ex vivo conditions allow experimentation on cells or tissue under more controlled conditions than in vivo experiments.

Histopathology: The microscopic examination of tissue, indicating disease.

In vivo: Experiments or measurements done on an intact organism.

Morphology: The study of the form and structure of organisms and their specific structural features.

Thalamus: A part of the vertebrate brain made up of two halves deep in the middle of the brain. Among other things, it is involved in relaying sensory and motor signals to the cerebral cortex, and regulating consciousness, sleep, and alertness.

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Cognitive Neuroscience Approaches to Understanding Behavior Change in Alcohol Use Disorder Treatments

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Researchers have begun to apply cognitive neuroscience concepts and methods to study behavior change mechanisms in alcohol use disorder (AUD) treatments. This review begins with an examination of the current state of treatment mechanisms research using clinical and social psychological approaches. It then summarizes what is currently understood about the pathophysiology of addiction from a cognitive neuroscience perspective. Finally, it reviews recent efforts to use cognitive neuroscience approaches to understand the neural mechanisms of behavior change in AUD, including studies that use neural functioning to predict relapse and abstinence; studies examining neural mechanisms that operate in current evidence-based behavioral interventions for AUD; as well as research on novel behavioral interventions that are being derived from our emerging understanding of the neural and cognitive mechanisms of behavior change in AUD. The article highlights how the regulation of subcortical regions involved in alcohol incentive motivation by prefrontal cortical regions involved in cognitive control may be a core mechanism that plays a role in these varied forms of behavior change in AUD. We also lay out a multilevel framework for integrating cognitive neuroscience approaches with more traditional methods for examining AUD treatment mechanisms.

Key words: Alcohol use, abuse, and dependence; alcohol use disorder; neuroscience; cognitive neuroscience; brain; cognition; neural mechanisms; pathophysiology; behavior change; behavioral intervention; relapse; abstinence; treatment

Understanding the mechanisms that underlie recovery from alcohol use disorder (AUD) is critical to advancing AUD treatment science (Huebner and Tonigan 2007; National Institute on Alcohol Abuse and Alcoholism [NIAAA] 2009). Scientific progress over the last three decades has led to the development of a number of effective behavioral and pharmacological AUD interventions (Dutra et al. 2008). However, even evidence-based treatments are only modestly effective. For example, reported rates of nonresponse to treatment interventions in major AUD treatment studies have ranged from 30 percent to 85 percent (Anton 2006; Johnson et al. 2007; McKay 2009; Project MATCH Research Group 1997). There is a general consensus that improving AUD behavioral intervention outcomes requires an understanding of the mechanisms that underlie behavior change in effective treatments (Magill and Longabaugh 2013; Morgenstern and McKay 2007). Thus, building a strong foundation for AUD treatment science includes answering the question of how, not just whether, a treatment is effective (Kazdin 2007).

To date, research on the mechanisms of effective AUD treatments that underlie behavior change have made limited progress, suggesting the need for major revisions in the theory and methods used for this work. Cognitive neuroscience may provide the tools for those revisions. Indeed, the pathophysiological processes that maintain AUD, such as craving, relapse, and withdrawal, are increasingly being understood in terms of the functioning of specific neural systems. As such, any psychosocial treatment for AUD that effectively changes behavior must interact at some level with these processes and, therefore, must influence these same neural systems. This article will review what cognitive neuroscience can tell us

about the neural bases of AUD and the mechanisms by which psychosocial treatments may function to elicit behavior change in AUD patients.

Psychosocial Treatment Mechanisms Research in AUD

There is a relatively large research literature on AUD behavioral treatment mechanisms (Huebner and Tonigan 2007; Longabaugh et al. 2013). This research largely represents an extension of assumptions and methods used to test treatment efficacy (Kazdin and Nock 2003; Morgenstern and McKay 2007; Wampold 2001). It has tested the treatment theories that guide evidence-based treatments using a set of mediation analysis procedures embedded within a clinical trials framework (Nock 2007). Stated succinctly, treatment theories postulate that the treatments work via some unique ingredient, often referred to as a specific effect—that is not present in other treatments (Morgenstern and McKay 2007). For example, theories postulate that motivational interviewing (MI) increases patients' motivation to change their behavior (Miller and Rose 2009) and that neither a weak control condition like psychoeducation nor even a bona fide effective treatment like 12-step facilitation affects a patient's motivation to change (Slaymaker and Sheehan 2013). Unfortunately, reviews of this literature generally conclude that there is limited support for most AUD treatment theories (Apodaca and Longabaugh 2009; Morgenstern and McKay 2007; Longabaugh et al. 2013). Indeed, most effective evidence-based AUD behavioral interventions yield equivalent outcomes even among subgroups where one would expect to find a difference. For example, MI typically has not proven superior to other AUD treatments among individuals with low motivation to change (Morgenstern and McKay 2007).

Even in instances where tests do not involve comparing treatments, it has often been difficult to establish seemingly straightforward links between treatment mediators and outcome. For example, Kelly and colleagues (2014) examined whether changes in peer networks mediated improved outcomes in 12-step treatment for young adults. Findings indicated that peer networks changed in the expected direction: posttreatment participants had fewer friends who used substances and more friends who abstained. Both greater affiliation with self-help organizations and changes in peer networks predicted improved outcome. However, contrary to prediction, the link between greater self-help affiliation and improved outcome was not mediated by changes in social networks. The authors concluded that more needs to be understood about how affiliation with self-help works to improve outcomes among youth with AUD.

It is important to note that some AUD treatment mediation studies have yielded important positive findings. For example, Moyers and colleagues (2009) found that improved outcomes in MI were mediated by increases in client motivational statements during treatment sessions. In addition, studies have consistently found that expected mediators such as motivation to change, self-efficacy, and social support for abstinence predict treatment outcome as well as improve during treatment, even though support for full mediation or specific effects generally has been absent. Overall, mediation analysis research has yielded less insight than expected about how AUD behavioral treatments work (Longabaugh et al. 2013). Given the relatively limited progress to date, it seems likely that major revisions in the theory and methods used to understand mechanisms of behavior change in AUD will be needed to advance this critical area of inquiry.

A major challenge to improving the informative value of AUD treatment mechanisms research is identifying the right measures to index the psychological processes that are hypothesized to mediate behavior change. Most of the conceptual frameworks and methods used to examine AUD treatment processes have not been revised to incorporate recent major conceptual and methodological advances for understanding the motivational, cognitive, affective, and, ultimately, neural processes that promote behavior change (Morgenstern et al. 2013). For example, constructs such as "motivation for change," "peer networks," or "coping skills" are very complex, and self-report measures designed to index them may encompass multiple psychological processes, some of which may relate to behavior change and others which may not. Furthermore, behavior change may depend upon psychological processes that are largely outside of conscious awareness and therefore not accessible by self-report measures. Moreover, such constructs may be difficult to relate to the underlying pathophysiology of addiction, which is understood increasingly in terms of highly specific affective, motivational, cognitive and neural processes. Cognitive neuroscience may hold the key to allowing researchers to use all of the processes to examine psychosocial treatment mechanisms.

Why Use Cognitive Neuroscience Approaches?

There are several reasons why understanding psychosocial treatment mechanisms at the neural level will be critical for advancing AUD treatment. Any psychosocial treatments for AUD that are effective at changing behavior must interact at some level with the pathophysiological processes that maintain AUD, which themselves are being understood increasingly in terms of the functioning of specific neural systems. Indeed, identifying neural systems that play a role in behavior change in psychosocial treatments can help researchers hone current treatments and develop more effective ones. For example, it can

facilitate more effective integration of behavioral treatments with medications, a goal that so far has proven elusive using purely clinical approaches (Combine Study Research Group 2006). In addition, measuring the functioning of brain systems involved in behavior change in a given treatment, especially when combined with genetic biomarkers, may be used to identify patients who are likely to respond to that treatment, another goal that has been elusive using purely clinical approaches (Project MATCH Research Group 1997). Other mental disorders that commonly co-occur with AUD, such as mood and anxiety disorders, also are now being understood in terms of the functioning of specific neural systems.

Among neuroscience approaches, cognitive neuroscience approaches have the most value for understanding psychosocial treatment mechanisms. Cognitive neuroscience approaches include a number of different methods aimed at understanding the relationship between relatively complex behaviors such as memory, attention, language, emotion and decisionmaking, and the structure and function of large-scale neural systems over relatively brief time periods (seconds). At a pragmatic level, cognitive neuroscience methods, such as structural and functional magnetic resonance imaging, allow for the noninvasive study of neural functioning in human subjects, which is critical in patient-oriented translational research. Also, compared with molecular or cellular approaches, the constructs addressed by cognitive neuroscience are nearer to the clinical phenomenology of AUD, as well as to the psychological constructs that have thus far been used to explain mechanisms of behavior change in AUD treatment.

Although cognitive neuroscience approaches may address certain clinically relevant questions that may improve the efficacy of psychosocial treatments, there is nothing inherently more valid or true about the neural level of understanding treatment mechanisms. A framework that integrates across multiple levels of analysis—social, interpersonal, behavioral, cognitive, and neural—will ultimately yield the most clinically useful understanding of behavior change. This would bring AUD research in line with the overall shift in mental health research to understand mental disorders and their treatments using a multilevel framework that includes neuroscience approaches (National Institute of Mental Health 2013).

Neurocognitive Models of Addiction Pathophysiology

Arguably, more is known about the pathophysiology of AUD and other substance use disorders than of any other mental disorders. This is in large measure attributed to the development of highly valid animal models of drug and alcohol addiction that mimic the basic elements of human addiction, including drug self-administration, conditioned-place preference, and cued relapse. Researchers have coupled these animal models with invasive methods for measuring and manipulating neural function with a high degree of spatial and temporal localization in order to provide a detailed picture of the neural mechanisms that maintain addiction. The consensus that has emerged from this extensive body of work, reviewed at length elsewhere (Everitt and Robbins 2005; Koob and Le Moal 2001; Robinson and Berridge 2008), is that drugs and alcohol trigger dopamine-induced sensitization within incentive neural systems, in particular the ventral striatum, which normally motivate and guide the seeking of natural rewards but, after being sensitized, come to motivate and guide the seeking of drugs and alcohol.

In parallel with this animal literature, a large number of functional imaging studies in patients with substance use disorders have revealed neural systems whose activity is increased by exposure to drug and alcohol cues. Schacht and colleagues (2013) conducted a recent meta-analysis of functional magnetic resonance imaging (fMRI) studies in which AUD patients were exposed to alcohol-related cues. Their analysis showed that, consistent with animal models, alcohol cues reliably elicit neural activation in the ventral striatum. It also showed that alcohol cues elicit activation in cortical regions involved in decisionmaking, cognitive control, and emotional experience, such as the ventromedial prefrontal cortex, the anterior cingulate cortex, and the insula. Importantly, the analysis found that the ventral striatum was the region in which activity was most consistently related to behavioral and self-report measures of alcohol seeking, such as craving, and in which treatment most consistently reduced activity.

More recent work has examined the role of prefrontal cortical systems in various inhibitory, cognitive control, and decisionmaking functions that moderate or shape alcohol-seeking motivation in the service of long-term goals and the avoidance of negative consequences. A number of studies have shown that AUD is associated with structural and functional abnormalities in the prefrontal cortex (Goldstein et al. 2004; Volkow et al. 1994), along with neuropsychological impairments in a variety of executive functions mediated by the prefrontal cortex (Sullivan et al. 1993, 1997). Bechara and colleagues (2000), for example, have found a critical role for the ventromedial prefrontal cortex in the successful performance of behavioral tasks that require the forgoing of short-term, but certain, rewards to avoid long-term, but uncertain, negative consequences. Subsequently, they demonstrated that AUD patients show impairments on these same behavioral tasks, similar to impairments seen in patients with ventromedial prefrontal cortex damage (Bechara and Damasio 2002; Bechara et al. 2002). The decisions in these tasks resemble an AUD patient's decision to abstain or relapse, which is a decision

to obtain a short-term reward (alcohol) without regard to a variety of uncertain, long-term negative consequences. Additionally, fMRI studies have linked dysfunction in the dorsolateral prefrontal cortex to impaired inhibitory control in AUD (Li et al. 2009). One study (Field et al. 2007) has linked AUD with impairments in delayed discounting and executive attention functions, both of which depend upon prefrontal cortical regions. A more recent study (Naqvi et al. 2015) finds that, compared with social drinkers, AUD patients are less able to reduce cue-induced craving by thinking about long-term negative consequences of alcohol use. This ability is a cognitive regulation function that fMRI studies in cigarette smokers show depends upon functional interaction between the dorsolateral prefrontal cortex and the ventral striatum (Kober et al. 2010).

Together, this work suggests that AUD is maintained by the interaction of two neural adaptations that arise as a result of chronic alcohol use:

- The dopamine-induced sensitization of the ventral striatum to alcohol and alcohol-related cues, leading to enhanced emotional and behavioral reactivity to these stimuli; and

- Impairments in prefrontal cognitive control functions, leading to an inability to regulate emotional and behavioral hyperreactivity to alcohol and alcohol-related cues that are driven by a sensitized ventral striatum.

These neural adaptations make it difficult for AUD patients to control alcohol use in the face of negative consequences, a hallmark of AUD. If this model is correct, then effective treatments for AUD should either directly downmodulate the ventral striatum reactivity to alcohol and alcohol-related cues, or they should enhance the prefrontal cortex's ability to regulate ventral striatal reactivity to alcohol and alcohol-related cues according to long-term goals and consequences.

Neurocognitive Predictors of Relapse

If AUD patients remain abstinent after they stop drinking, it suggests that the behavior change mechanisms of their treatment worked. Conversely, if they relapse after a period of abstinence, it suggests that the same behavior change mechanisms failed. Thus, it may be possible to infer mechanisms of behavior change by identifying neural measures that predict relapse and abstinence. One of the first studies to do this, by Wrase and colleagues (2008), measured regional brain volumes in several reward-related brain regions in detoxified AUD patients. They found that the volume of the amygdala was lower in patients who relapsed to heavy drinking by 6 months, compared with those who abstained. Subsequently, Cardenas and colleagues (2011; Durazzo et al. 2011) showed that, compared with patients who abstained, patients who relapsed by 8 months posttreatment had relatively smaller total volume in the orbitofrontal cortex. Similarly, Rando and colleagues (2011) showed that patients with a smaller volume of gray matter in medial prefrontal regions, including the anterior cingulate cortex, relapsed more quickly and were more likely to drink heavily during relapse than patients with larger gray-matter volumes. What is not clear from these studies is whether a reduction in volume represents a loss of function, which would tend to increase relapse risk in the case of prefrontal cognitive control systems that regulate alcohol seeking, or whether the reductions represent a gain of function, which would tend to increase relapse risk in the case of incentive motivational systems that promote alcohol seeking.

These limitations may be addressed by functional imaging studies that examine how neural activity measured under various conditions predicts relapse. Several of these studies have been completed to date:

- Seo and colleagues (2013) measured neural activity during alcohol cue exposure, stressful imagery, and neutral imagery. They found that activity in the ventromedial prefrontal cortex and anterior cingulate cortex during neutral imagery predicted relapse within 3 months.

- In a small study, Braus and colleagues (2001) showed that alcohol cue–elicited activity in the ventral putamen predicted relapse within 3 months.

- Grusser and colleagues (2004) showed that alcohol cue–elicited activity in the putamen, anterior cingulate, and adjacent medial prefrontal cortex predicted relapse at 3 months.

- Heinz and colleagues (2007) failed to show a correlation between alcohol cue–elicited neural activity and relapse within 6 months but did show that neural activity elicited by positive emotional pictures within the thalamus and ventral striatum predicted abstinence.

- Camchong and colleagues (2013) showed that lower resting-state connectivity between "reward" and "executive control" regions during early abstinence predicted relapse within 6 months. They also found that resting-state connectivity between these systems was negatively correlated with poor inhibitory control in an affective go/no-go task.

Many of these functional imaging studies did not address patients' engagement in informal treatments such as 12-step groups during the follow-up period. This limitation makes it unclear whether neural activity was predictive of "intrinsic" abstinence capabilities,

or of the capacity to respond to these informal treatments. That said, together, these structural and functional imaging studies point toward neural systems that promote abstinence that already has been initiated. As such, they may not be generalizable to understanding the neural mechanisms by which actively drinking AUD patients reduce their alcohol use. This may bear upon the distinction between treatments intended to prevent relapse and treatments intended to initiate abstinence or to moderate alcohol use. Moreover, it is not clear whether results of studies examining predictors of abstinence and relapse in nontreatment samples can even be generalized to understand behavior change that results from effective treatments. This will require studies that examine neural functioning in treatment-seeking AUD patients both prior to and after completing treatment.

Neurocognitive Mechanisms of Existing, Evidence-Based AUD Treatments

A small number of studies have attempted to examine the specific neurocognitive mechanisms by which existing effective behavioral interventions change behavior, a concern that is central to mechanisms of behavior change initiation (MOBC) research (NIAAA 2009). In one study, Vollstädt-Klein and colleagues (2011) used fMRI to examine changes in neural activity elicited by alcohol-related cues both before and after participants received nine sessions of cue-exposure treatment (CET), which was added to supportive outpatient treatment. The researchers compared these patients with patients who received supportive outpatient treatment alone. They found that patients receiving CET showed a greater reduction in cue-elicited activity in the ventral and dorsal striatum, the anterior cingulate cortex, the precentral gyrus, the insula, and several prefrontal regions. This finding is consistent with a reduction in the rewarding interoceptive effects of alcohol as a result of CET.

DeVito and colleagues (2012) used fMRI to examine changes in neural activity related to the Stroop color–word interference task, which engages cognitive control and executive attention functions, in patients with substance use disorders that included AUD. Patients performed the Stroop task during fMRI both before and after receiving treatment. Half of the patients received treatment as usual from an outpatient drug treatment program along with 8 weeks of biweekly computerized cognitive behavioral therapy (CBT). The other half only received treatment as usual. Study authors found that patients receiving CBT improved their performance on the Stroop task and had decreased task-related activity in the anterior cingulate cortex (ACC), inferior frontal gyrus, and the midbrain. This is consistent with the theory that CBT improves general cognitive control functions. The study did not examine whether CBT changed neural activity related to alcohol-specific cognitive control functions, such as performance on an alcohol-specific Stroop task or cognitive regulation of alcohol craving, which would speak more specifically to the mechanisms of changing alcohol use behavior, as opposed to general self-regulatory mechanisms. Furthermore, this study did not examine AUD specifically but rather grouped patients with AUD with patients with other substance use disorders.

In another fMRI study, Feldstein Ewing and colleagues (2011) compared neural responses with alcohol cues during exposure to "change talk" and "counterchange talk," which are linguistic/semantic constructs hypothesized to mediate behavior change in MI. Study participants were AUD patients seeking treatment. The study found that exposing patients to alcohol-related cues while they listened to counterchange talk elicited activity in the ventral striatum, orbitofrontal cortex, and insula, whereas none of these areas showed any activity during change talk. These regions all play a role in representing the incentive value of rewards. This suggests that change talk may downmodulate the neural representations of the incentive value of alcohol-related cues. The study did not examine how these responses changed over the course of MI treatment, which would be necessary to infer whether this mechanism actually plays a role in this particular treatment.

These studies are important first steps; however, they possess a number of limitations. For example, none of them reported drinking outcomes after the interventions, which limits the ability to infer whether changes in neural functioning due to the interventions drive behavior change. Also, the control interventions were not themselves effective treatments that were missing only the hypothesized behavior change mechanism. This is important because existing evidence-based AUD treatments are complex, with multiple psychological components, many of which potentially affect behavior. This makes it necessary to examine neural mechanisms of behavior in existing treatments in a "top-down" fashion by decomposing complex intervention-specific constructs, such as change talk and coping skills into specific neurocognitive functions, such as reversal learning, cognitive control, emotion regulation, and response inhibition, both as they relate to alcohol and as they relate to general reward functions.

Novel AUD Treatments Derived From Neurocognitive Mechanisms

An alternative approach to understanding behavior change in AUD involves constructing novel interventions based upon our current understanding of the neurocognitive mechanisms of AUD pathophysiology and behavior change. As discussed above, AUD is associated with impairments in a number of executive functions that require regulation of subcortical reward-related and automatic processes by

prefrontal regions, including working memory, inhibitory control, reward learning, and craving regulation. Thus, interventions targeted at remediating these impairments should lead to reductions in alcohol use behavior. This provides both a new set of effective treatments and also indirectly tests hypotheses about the role of cognitive functions that are being remediated and, by extension, their neural substrates, in behavior change.

In a study by Houben and colleagues (2011*b*), non–treatment-seeking heavy drinkers completed 25 daily sessions of general working-memory training, including tasks designed to improve digit span, letter span, and visual-spatial working memory, all with progressively increasing difficulty. A heavy-drinking control group performed similar tasks that did not increase in difficulty. Participants in the active intervention group had improved working-memory function and, more importantly, significantly reduced the number of drinks they drank per week, compared with participants in the control group. This effect persisted for more than a month. The researchers also collected data on participant performance on an implicit alcohol association test, which measures the automaticity of processing alcohol-related information. They found that changes in working-memory capacity mediated the effects of working-memory training on reduction in alcohol use and that baseline performance on the implicit association test moderated this relationship. These findings provide circumstantial evidence that working-memory training reduced drinking by increasing control over automatic alcohol-related processing.

In another study, Houben and colleagues (2011*a*) examined the effect of a different cognitive task on non–treatment-seeking heavy drinkers. In a single session, one group of participants learned to provide "go" responses to non–alcohol-related cues and "no-go" responses to alcohol-related cues. Another heavy drinking group completed a version of the task requiring "go" responses to alcohol cues and "no-go" responses to nonalcohol cues. The researchers found that subjects in the no-go alcohol group significantly reduced their drinking in the week after the task, whereas subjects in the go alcohol group increased their drinking. Performance on this kind of go/no-go paradigm depends upon inhibitory control as well as reward-learning functions, suggesting that such functions may play a role in behavior change in AUD. However, this study did not provide a direct test of this model.

Both of these studies were relatively small and were undertaken in non–treatment-seeking heavy drinkers, as opposed to treatment-seeking patients diagnosed with AUD. Therefore, it is not known if these interventions would have similar effects in more severe, treatment-seeking AUD populations, who generally have more severe drinking problems and are likely to have a higher level of dysfunction in the neurocognitive functions being addressed by these interventions. It also is possible that the effects of these interventions were small, compared with potential effects of entering into a formal treatment with a high level of motivation for change, as is the case with many treatment seekers.

A larger study by Wiers and colleagues (2011) addressed these limitations. The study examined the effect of cognitive-bias modification (CBM) given to AUD patients prior to entering inpatient rehabilitation. CBM involved training patients to push a joystick away (an avoidance movement) whenever they saw an alcohol cue. This intervention is similar to the go/no-go task in that it involves repeatedly assigning a negative value (in this case a movement with intrinsic negative valence) to alcohol. Participants in the control groups received either no training or a training condition in which they had to make equal numbers of avoidance movements to alcohol cues and nonalcohol cues. The researchers followed patients for a year after they completed inpatient rehabilitation. The results showed that patients who received CBM prior to entering inpatient rehabilitation were somewhat less likely to relapse. And although the effect was just below the threshold for statistical significance, it provides circumstantial evidence that such implicit forms of reappraisal of alcohol's value may affect behavior change.

Summary and Limitations of Cognitive Neuroscience Approaches

A theme that emerges from the disparate lines of research reviewed here is that effective treatments for AUD serve to increase prefrontal cortex function and downmodulate the function of reward systems, especially the ventral striatum. Given the role of functional interactions between the prefrontal cortex and the ventral striatum in a variety of self-regulation processes (Ochsner et al. 2012), it is likely that increased functional interaction between these regions may serve as a critical behavior change mechanism that is shared by a number of different effective psychosocial treatments. In other words, findings from cognitive neuroscience predict that effective treatments increase prefrontal cortical function, decrease ventral striatal function, and increase functional connectivity between these two regions, especially during the processing of alcohol-related information (figure 1). Although a number of the studies cited here provide circumstantial evidence for this mechanism, no studies have tested it directly.

Another important theme that emerges from this literature is whether behavior change mechanisms related to AUD are specific to alcohol use or more general cognitive changes. AUD is associated with deficits in a number of general cognitive functions, especially executive and cognitive control functions, as well as specific "gains of function," with respect to the incentive and rewarding effects of alcohol and related cues. Thus, it is important to understand whether a given intervention changes alcohol use behavior

because it influences general cognitive functions or because it influences functions that are specific to the processing of alcohol-related information. For example, it is possible that interventions aimed at reducing the incentive salience of alcohol cues, such as cue-exposure therapy, and interventions aimed at increasing the ability to specifically regulate this incentive salience, such as cognitive bias modification and cognitive regulation of craving, are mediated by the specific mechanism of prefrontal executive/cognitive control regions modulating the processing of alcohol's incentive value by subcortical reward-related regions. Concurrently, interventions aimed more generally at improving prefrontal cortex functions, such as working-memory training, may facilitate the more specific interventions because these general functions play a part in alcohol-specific regulation functions.

Although cognitive neuroscience approaches provide a window into AUD treatment mechanisms that aligns with our current understanding of AUD pathophysiology, there are limitations to cognitive neuroscience approaches that affect the ability to infer AUD treatment mechanisms. A major limitation of all functional imaging studies is that they are essentially correlational. Merely showing that a given psychological process is associated with increased activity within a specific neural system does not by itself prove that this neural system is critically necessary for the psychological process. By extension, merely showing that neural activity within a brain system changes as a result of a treatment does not demonstrate that this treatment must affect this brain system to elicit behavior change. When examining disease pathophysiology, it is difficult to know whether differences between patients and healthy controls in brain structure and function play a causal role in disease pathology, whether they are merely parallel phenomena, or whether they pre-exist disease development. This issue may be addressed in prospective studies in at-risk individuals (see Ersche et al. 2012 for an example of this approach applied to structural brain abnormalities in addiction). Such limitations are not specific to AUD treatment research; they are inherent in all translational neuroimaging studies that aim to examine pathophysiology and treatment mechanisms.

Future Directions

Using cognitive neuroscience approaches to study behavior change in psychosocial treatments for AUD is a young field.

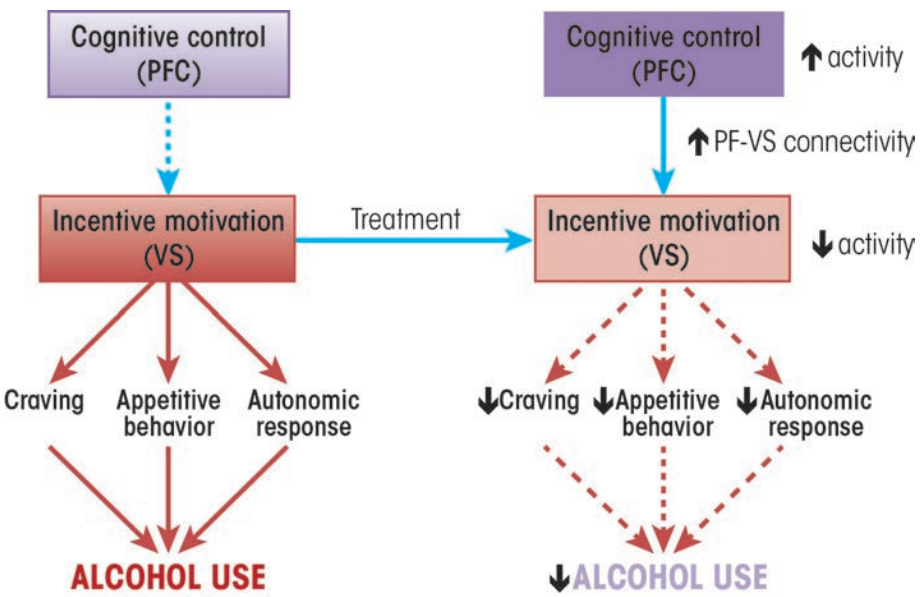

Figure 1 A potential common mechanism for alcohol use disorder (AUD) treatments. A number of studies suggest that AUD treatments elicit behavior change by increasing the regulation of brain regions that mediate incentive motivation, such as the ventral striatum, by prefrontal cortical regions that mediate cognitive control. Arrows denote expected changes in specific neural, behavioral, psychophysiological and clinical outcome measures, given this hypothesized treatment mechanism. PFC = prefrontal cortex. VS = ventral striatum.

Future studies can address some of the current weaknesses of this field by integrating cognitive neuroscience approaches with the conceptual and methodological approaches that already have proven useful for examining AUD treatment mechanisms. The first step in such an approach is to identify specific cognitive, affective, and behavioral processes that are hypothesized to mediate behavior change in a given treatment. The next step is to operationalize these processes using relatively simple paradigms that can be implemented in functional imaging experiments. This also should include appropriate control tasks that are ideally the same as the experimental tasks, minus the psychological processes under study. There should be preliminary data showing which neural parameters (i.e., activity measures in specific brain systems, along with measures of connectivity between brain systems) are changed by this task, compared with the control task, and how this relates to behavioral measures acquired during the functional imaging experiments. There should also be a clear set of a priori hypotheses about which of these neural parameters relate to behavior change in the treatment and which do not. The clinical population should be well characterized using self-report measures of AUD severity and or psychological processes that have already been studied as mediators of behavior change in the treatment under study. Patients should be randomly assigned to receive the active treatment or an equally effective control treatment that is hypothesized to not depend upon the processes under study. Functional imaging data, along with self-report measures, should be acquired both prior to and then immediately following the treatments. Appropriate clinical outcome measures should be specified.

What kind of results would be necessary to support the role for a specific neural system in the mechanism of a treatment? First, it would be necessary to show that the active treatment, but not the control treatment, changed the functioning of this neural system as it relates to the specific psychological process under study. Second, it would be necessary to show that the relationship between the treatment and the clinical outcome was statistically mediated by the effect of treatment on the functioning of this neural system. Third, it would be useful to relate changes in neural function from pre- to posttreatment to changes in self-report measures indexing psychological processes already known to mediate behavior change in the

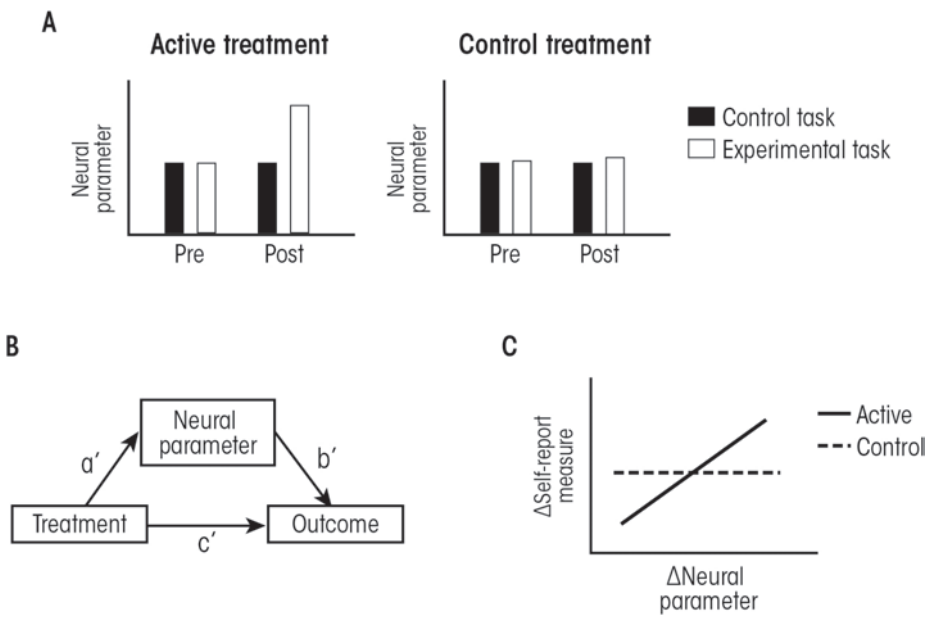

Figure 2 Predicted results from experiments directed at addressing the role of neural systems in alcohol use disorder (AUD) treatment mechanisms. **(A)** An active treatment should increase the neural parameters that index the functioning of these systems as it relates to a specific psychological process of interest (the experimental task). There should be no effect of the control treatment on these neural parameters. **(B)** The effects of a treatment on the neural parameter should mediate the effects of the treatment on clinical outcome. **(C)** Changes (Δ) in the neural parameters from pre- to posttreatment should correlate with corresponding changes in self-report measures that index psychological processes already known to drive behavior change.

treatment. This would help to clarify whether the neural system plays a role in psychological processes already known to be involved in behavior change, or whether neural systems impact some other, as yet unknown, psychological processes that drive behavior change. This approach is illustrated in figure 2.

Once a neural system is identified as playing a role in behavior change in a specific treatment, additional studies can use "interventional" approaches, such as transcranial magnetic stimulation, to examine how noninvasively disrupting or enhancing the functioning of this neural system impedes or augments behavior change during the treatment. Additionally, researchers can add medications that are known to target this neural system to the treatment, and observe the effect on behavior change. Researchers also can seek out AUD patients who acquire brain damage in the neural system—for example from a stroke—and examine whether the brain damage reduces the efficacy of the treatment as a result of impairments in the psychological processes mediated by the damaged neural system. These approaches would provide direct tests of the role of the neural system and the psychological processes it mediates in behavior change, as opposed to the correlational evidence provided by functional neuroimaging.

Although such an approach attempts to relate changes in neural parameters acquired in functional imaging experiments to changes in behavior, it is important to note that the neural parameters by themselves do not constitute a mechanism. Rather, they are measurements of the functioning of specific neural systems that are involved in psychological processes that drive behavior change. In this way, the approach must integrate across multiple levels of analysis. Such an integrative approach does not place a higher value on neural measures compared with psychological or clinical measures. Instead, the approach depends on several levels of analysis in order to arrive at a coherent, clinically useful understanding of how currently effective treatments change behavior, one that can ultimately facilitate the development of novel, more effective treatments.

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Circadian Disruption

Potential Implications in Inflammatory and Metabolic Diseases Associated With Alcohol

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Circadian rhythms are a prominent and critical feature of cells, tissues, organs, and behavior that help an organism function most efficiently and anticipate things such as food availability. Therefore, it is not surprising that disrupted circadian rhythmicity, a prominent feature of modern-day society, promotes the development and/or progression of a wide variety of diseases, including inflammatory, metabolic, and alcohol-associated disorders. This article will discuss the influence of interplay between alcohol consumption and circadian rhythmicity and how circadian rhythm disruption affects immune function and metabolism as well as potential epigenetic mechanisms that may be contributing to this phenomenon. **KEY WORDS: Alcohol consumption; alcohol-related disorders; disease factors; risk factors; circadian disruption; circadian rhythm; circadian clock; immune function; metabolism; inflammatory diseases; metabolic diseases; epigenetic mechanisms**

Circadian Disruption and Society

The circadian clock is a sophisticated mechanism that functions to synchronize (i.e., entrain) endogenous systems with the 24-hour day in a wide variety of organisms, from simple organisms such as fungi up to the complex mammalian systems. Circadian rhythms control a variety of biological processes, including sleep/wake cycles, body temperature, hormone secretion, intestinal function, metabolic glucose homeostasis, and immune function. Functional consequences of modern-day society, such as late-night activity, work schedules that include long-term night shifts and those in which employees change or rotate shifts (i.e., shift work), and jet lag are substantial environmental disruptors of normal circadian rhythms. Fifteen percent of American workers perform shift work (Bureau of Labor Statistics 2005), indicating the pervasiveness of circadian disruption as a normal part of modern-day society. This change from the diurnal lifestyle of our ancestors to one that is more prominently nocturnal results in misalignment between natural rhythms based on the 24-hour day and behavioral activity patterns (i.e, circadian misalignment). Circadian misalignment has a significant detrimental effect on cell, tissue, and whole-organism function. These alterations can manifest in humans as chronic health conditions, such as metabolic syndrome,[1] diabetes, cardiovascular disease, cancer, and intestinal disorders (Karlsson et al. 2001; Morikawa et al. 2005; Schernhammer et al. 2003; Penev et al. 1998; Caruso et al. 2004). The increased prevalence of diseases associated with circadian disruption underscores the need to better understand how circadian disruption can wreak havoc in so many different ways throughout the body.

[1] Metabolic syndrom is a combination of disorders that can lead to diabetes and cardiovascular disease, characterized by abnormal levels of fat and/or cholesterol in the blood and insulin resistance.

Central and Peripheral Circadian Rhythms

The master or central circadian clock (i.e., "pacemaker") is located in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus in the brain (Turek 1981) (see figure 1). The SCN is regulated by light stimulating retinal ganglion cells in the eye (Berson et al. 2002), and it is by this mechanism that light directs central circadian rhythms. Circadian rhythms are found in nearly every cell in the body, including the periphery, encompassing the immune system, heart, adipose tissue, pancreas, and liver (Allaman-Pillet et al. 2004; Boivin et al. 2003; Storch et al. 2002;

Yoo et al. 2004; Zvonic et al. 2006). The SCN synchronizes circadian rhythms found in the periphery (figure 2A) via several mechanisms, including communication with nerve cells that influence visceral functions such as digestion, heart rate, etc., via direct release of the hormones oxytocin and vasopression into the general vasculature or indirectly via release of local signals that affect the release of hormones from the anterior pituitary gland (i.e., neuroendocrine and autonomic neurons) (Buijs et al. 2003). In addition, peripheral circadian rhythms can be regulated by external factors other than central light-entrained rhythms. For instance, abnormal feeding patterns can cause peripheral circadian rhythms (i.e., in the intestine and liver) to become misaligned with central rhythms if feeding is out of synch with the normal 24-hour pattern, a phenomenon that can be observed in both animals and humans (see figure 2B). Peripheral tissues express self-sustained rhythms that are able to function independent of the central clock in the SCN. For example, following SCN lesion that terminates central circadian rhythmicity, peripheral circadian clocks continue to demonstrate rhythmicity; however, peripheral rhythms become desynchronized from each other over time (Yoo et al. 2004) (see figure 2C). This internal misalignment is particularly detrimental because peripheral circadian clocks directly regulate up to 5 to 20 percent of the genome (i.e., so-called clock-controlled genes) (Bozek et al. 2009). Furthermore, reports indicate that 3 to 20 percent of the entire genome demonstrates 24-hour oscillations in gene expression, including genes critical for metabolic processes. This observation suggests that although not directly controlled by the circadian clock, genes are influenced as a consequence of rhythmic changes in transcription factors and transcriptional (i.e., the process of creating a complementary RNA copy of a sequence of DNA) and translational (i.e., when RNA is used to produce a specific protein) modifiers (i.e., proteins controlling the levels and activity of various processes including lipid metabolism and glucose synthesis) (Panda et al. 2002).

At the cellular level, circadian rhythms originate from self-sustained, autoregulated, cyclic expressions of clock genes, which constitute the molecular clock. The molecular circadian clock consists of transcriptional activators and repressors—that is, proteins that stimulate and repress the production of proteins, respectively, in a cyclic process that is approximately 24-hours in duration (Reppert and Weaver 2002). The molecular circadian cycle is initiated when the transcriptional activators Clock and Bmal1 (Bunger et al. 2000) combine (i.e., heterodimerize) to stimulate the transcription of target circadian genes including period (*Per*) and cryptochrome (*Cry*) (i.e., *Per1* to *Per3* and *Cry1* and *Cry2*) as well as a host of other clock-controlled genes. When PER and CRY proteins accumulate in the cytosol, they heterodimerize and translocate to the nucleus where they act as transcriptional repressors to terminate CLOCK-BMAL1–mediated transcription, thus ending the molecular circadian cycle (van der Horst et al. 1999) (see figure 3). The cycle is further regulated by additional proteins, including the enzyme sirtuin 1 (SIRT1), a histone deacetylase that modifies circadian proteins or DNA by removing acetyl groups to alter gene expression. SIRT1 is sensitive to levels of the coenzyme nicotinomide adenine dinucleotide (NAD^+), making NAD availability a potential regulator of the molecular circadian clock (Grimaldi et al. 2009). The details of this oscillating cycle are found elsewhere (Reppert and Weaver 2002).

Demonstrating the importance of the molecular circadian clock, mutations of the core circadian clock components can have a devastating effect on the function of the circadian clock. This is true for both *Bmal1* (Bunger et al.

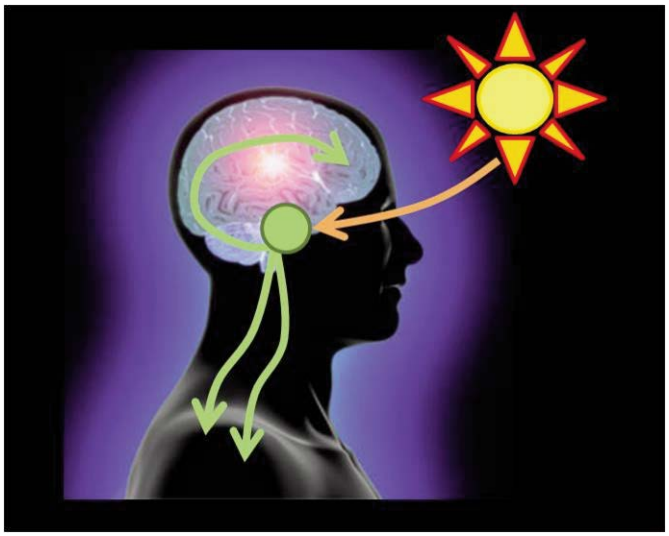

Figure 1 The suprachiasmatic nucleus (SCN) is the central circadian pacemaker. The SCN is located in the hypothalamus and is regulated by light signals from the eye. The SCN then affects a wide variety of physiological and behavioral outcomes.

2000) and *Clock* (Oishi et al. 2006). Likewise, molecular perturbation of the circadian clock (i.e., altering the *Clock, Bmal1, Per1, Per2, Cry1,* or *Cry2* expression via genetic manipulations including deleting or mutating the gene of interest to affect the levels of functional protein produced) disrupts normal circadian behavioral rhythms (Antoch et al. 1997; Bunger et al. 2000; van der Horst et al. 1999; Zheng et al. 2001). This article will discuss the influence of alcohol on circadian rhythms and how circadian-rhythm disruption affects immune function and metabolism, significant factors for alcohol-associated poor health outcomes. It also will discuss potential epigenetic mechanisms by which circadian disruption and alcohol may establish long-term changes in gene expression, resulting in adverse health outcomes.

Alcohol and Circadian Rhythmicity

Circadian organization and stable circadian rhythms are vital for optimal health as numerous diseases are associated with circadian-rhythm disruption. Environmental factors such as shift work or jet lag are obvious disrupters of circadian rhythmicity. However, other environmental factors, such as alcohol consumption and the timing of food intake, can profoundly disrupt and disorganize circadian rhythmicity, which can be observed on behavioral, cellular, and molecular levels.

Alcohol Disrupts Behavioral and Biological Circadian Rhythms

Alcohol has a dramatic effect on circadian rhythms. These circadian abnormalities include disrupted sleep/wake

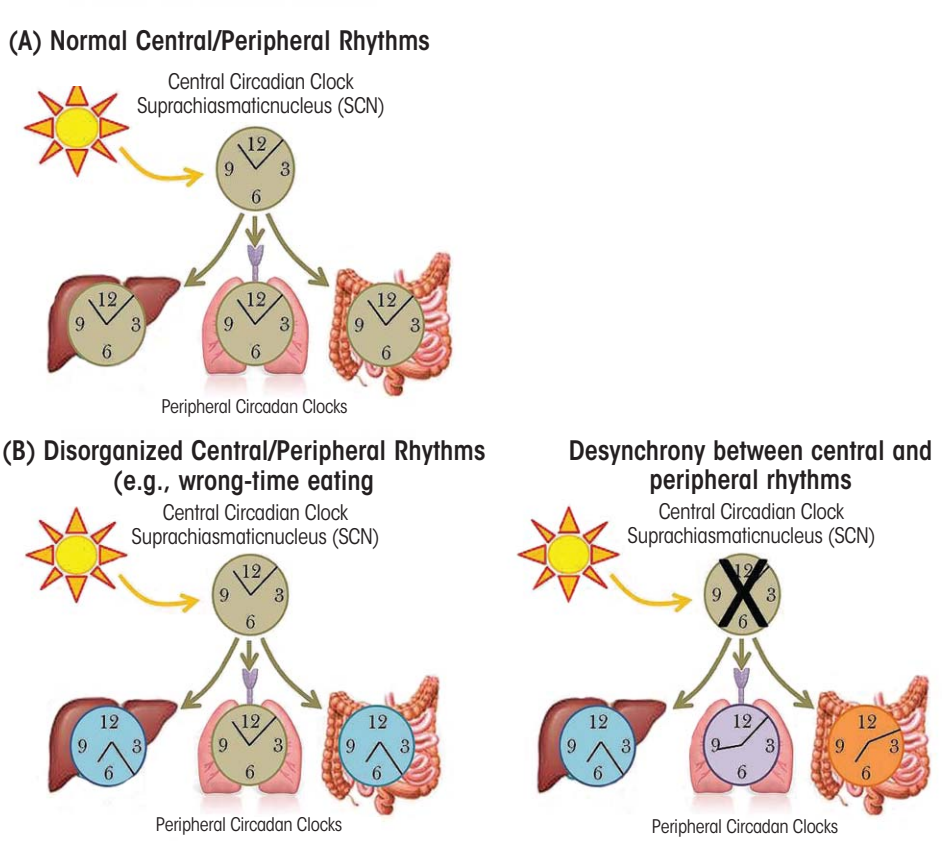

Figure 2 Central and peripheral circadian rhythms. **(A)** Under normal conditions, the central circadian clock in the suprachiasmatic nucleus which is entrained by light, then regulates peripheral circadian clocks. **(B)** Wrong-time eating can cause misalignment between the central circadian clock (entrained by light) and the peripheral circadian clocks entrained by food (illustrated here are intestine and liver). **(C)** When the central circadian clock is disrupted (e.g., due to lesion) peripheral circadian clocks will continue to cycle but will gradually become more misaligned with each other.

cycles in humans (Brower 2001; Imatoh et al. 1986) as well as disrupted circadian responses to light and abnormal activity patterns in rodents (Brager et al. 2010; Rosenwasser et al. 2005). The changes observed in behavioral patterns and responses to light may be the consequence of alcohol-induced disruption of normal tissue/organ function and neuroendocrine function. For example, normal cyclic patterns associated with body temperature (i.e., thermoregulation) (Crawshaw et al. 1998), blood pressure (Kawano et al. 2002), and characteristics of biochemical circadian rhythms including glucose and cholesterol rhythms (Rajakrishnan et al. 1999) are significantly affected by alcohol consumption. In addition, the circadian-driven production of hormones including melatonin (i.e., an endocrine hormone that is important in circadian entrainment) in rats (Peres et al. 2011) and humans (Conroy et al. 2012), corticosterone (i.e., a steroid hormone produced by the adrenal gland that responds to stress and regulates metabolism) (Kakihana and Moore 1976), and pro-opiomelanocortin (i.e., a polypeptide hormone that is a precursor to several hormones) (Chen et al. 2004) are disrupted by alcohol consumption. Alcohol-induced changes such as these have a profound impact on the functioning of a wide variety of peripheral organs and biological processes, which are dependent upon central circadian synchronization for proper function.

Alcohol Disrupts the Molecular Circadian Clock

Not surprisingly, the changes observed in the behavioral and biological systems also are observed on the molecular level as a disrupted molecular circadian clock, an effect that is evident both in vitro and in vivo. Exposure of intestinal epithelial cells (i.e., Caco-2 cells, a widely used model of the human intestinal barrier) to alcohol increases the levels of circadian clock proteins CLOCK and PER2 (Swanson et al. 2011). Likewise, alcohol-fed mice have disrupted expression of *Per1–Per3* in the hypothalamus (Chen et al. 2004), human alcoholics demonstrate markedly lower expression of *Clock, BMAL1, Per1, Per2, Cry1,* and *Cry2* in peripheral blood mononuclear cells (i.e., immune cells) compared with nonalcoholics (Huang et al. 2010), and in humans alcohol consumption is inversely correlated to BMAL1 expression in peripheral blood cells (Ando et al. 2010). The alcohol-induced changes seem to have long-lasting effects on the circadian clock, particularly when the exposure occurs early in life, which may be the consequence of epigenetic modifications (discussed below). For example, neonatal alcohol exposure in rats disrupts normal circadian-clock expression levels and expression patterns over a 24-hour period (i.e., rhythmicity) (Chen et al. 2006; Farnell et al. 2008). These examples illustrate the ability of alcohol to have profound and long-lasting effects on clock-gene expression in multiple organs and tissues.

Feed-Forward Cycle: Alcohol Promotes Circadian Disruption and Circadian Disruption Promotes Alcohol Consumption

Interestingly, circadian-clock disruption can promote alcohol consumption, which can further exacerbate this cycle. For example, *Per2* mutant mice exhibit increased alcohol consumption compared with wild-type counterparts (Spanagel et al. 2005), an effect attributed to altered reinforcement systems leading to enhanced motivation to consume alcohol. This may explain why humans with circadian disruption are more prone to substance abuse disorders (Trinkoff and Storr 1998). This phenomenon also sets up a potentially devastating cycle in which circadian disruption drives alcohol consumption, which further exacerbates circadian disruption.

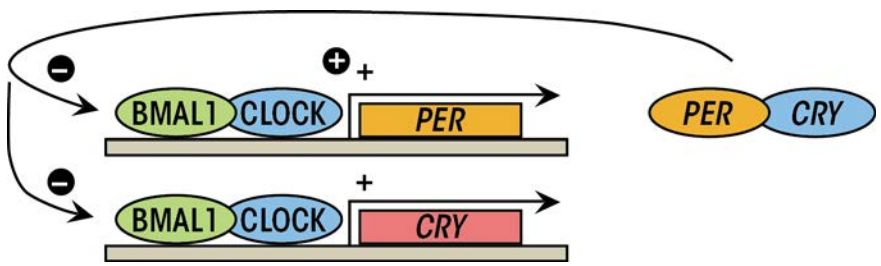

Figure 3 The molecular circadian clock. Transcription of the clock-controlled genes, including *Per* and *Cry* is initiated by the heterodimerization and binding of BMAL1 and CLOCK (the positive limb of the molecular circadian clock). Once sufficient amounts of PER and CRY have been produced, they dimerize and inhibit further BMAL1/CLOCK-mediated transcription (the negative limb of the molecular circadian clock).

Mechanisms of Alcohol-Induced Circadian Disruption

The mechanisms by which alcohol disrupts circadian rhythmicity are likely a consequence of alcohol metabolism and alcohol-induced changes in intestinal barrier integrity.

Consequences of Alcohol Metabolism

Alcohol is metabolized via several mechanisms, including the enzymes catalase, alcohol dehydrogenase (ADH), and cytochrome P450 (CYP2E1) (Lu and Cederbaum 2008). Although alcohol metabolism most prominently occurs in the liver, other tissues such as the stomach, intestine, and brain also play a role in this process. One consequence of alcohol metabolism that is particularly relevant for alcohol-induced disruption of circadian rhythmicity is a shift in the cellular NAD^+/NADH ratio. SIRT1, which regulates the molecular circadian clock, is highly sensitive to the cellular NAD^+/NADH ratio. Therefore, a perturbation in the availability of NAD^+ (e.g., as a consequence of alcohol metabolism by ADH or as a consequence of aldehyde metabolism by acetaldehyde) would be one mechanism by which alcohol could disrupt the molecular circadian clock and resulting circadian rhythms.

Alcohol, the Intestine, and Inflammation

Another mechanism by which alcohol can exert a negative influence on circadian rhythmicity is by promoting intestinal hyperpermeability. Alcohol disrupts intestinal barrier integrity in vitro (Swanson et al. 2011), in rodents (Keshavarzian et al. 2009), and humans (Keshavarzian et al. 1994, 1999). Intestinal hyperpermeability allows luminal bacterial contents such as endotoxin (e.g., lipopolysaccharide (LPS) to translocate through the intestinal epithelium into the systemic circulation. Endotoxin can disrupt circadian rhythms. LPS administered to rodents impairs the expression of *Per* in the heart, liver, SCN, and hypothalamus (Okada et al. 2008; Yamamura et al. 2010) and suppresses clock gene expression in human peripheral blood leukocytes (Haimovich et al. 2010). Thus, intestinal-derived LPS may be one mechanism by which alcohol disrupts circadian rhythmicity. In addition, LPS elicits a robust immune response in the periphery (Andreasen et al. 2008), and systemic inflammation disrupts normal circadian rhythmicity (Coogan and Wyse 2008). For example, tumor necrosis factor α (TNFα), a cytokine produced in response to endotoxins, disrupts normal locomotor behavior and sleep/wake cycles and alters expression of the molecular circadian clock in the liver (Cavadini et al. 2007). Thus, there are several plausible mechanisms by which alcohol-induced effects on the intestine may disrupt central and peripheral circadian rhythms.

Intestinal-derived LPS may be one mechanism by which alcohol disrupts circadian rhythmicity.

It is clear that alcohol-induced effects on the intestine are highly detrimental to circadian rhythmicity. Interestingly, the reverse also is true in that the molecular circadian clock in the intestine influences alcohol-induced effects. Intestinal circadian rhythms are largely driven by feeding patterns (Hoogerwerf et al. 2007; Scheving 2000) and even the apical junctional complex (AJC) proteins, which regulate tight junctions (and thus intestinal permeability), are clock controlled in the kidney (Yamato et al. 2010). Alcohol exposure increases intestinal circadian gene expression, and knocking out *Clock* or *Per2* in intestinal epithelial cells (i.e., Caco-2 cells) prevents alcohol-induced intestinal hyperpermeability (Swanson et al. 2011). Taken together, alcohol—via metabolism products or intestine effects including endotoxemia and systemic inflammation—disrupts intestinal circadian rhythms, an effect that can further exacerbate internal misalignment.

Circadian Rhythms and Immune Function

The immune system demonstrates robust circadian rhythmicity with daily variations in immune parameters, including lymphocyte proliferation, antigen presentation, and cytokine gene expression (Fortier et al. 2011; Levi et al. 1991). These rhythms seem to be sensitive to perturbations in circadian homeostasis, with differential effects depending on the cell type, model system, and outcome measure. For example, inhibition of *Per2* in natural killer (NK) cells (part of the innate immune system) decreases the expression of the immune effectors granzyme-B and porforin (i.e., critical cytotoxic components) (Arjona and Sarkar 2006*a*). Despite these changes, selective reduction of *Per2* in NK cells does not effect NK rhythmic production of the cytokine interferon-γ (IFNγ), which is important for the formation and release of reactive oxygen species. In contrast, whole-animal *Per2*-deficient mice have drastically disrupted IFNγ rhythms (Arjona and Sarkar 2006*b*). The IFNγ rhythmic disruption in *Per2*-deficient mice but not after selective reduction of *Per2* in isolated NK cells would be expected if IFNγ is dependent upon other circadian parameters, such as circadian fluctuations in hormones or temperature. Indeed, rhythmic hormones such as glucocorticoids and melatonin, which are significantly affected by circadian disruption, modulate immune function (Dimitrov et al. 2004; Srinivasan et al. 2005). *Per2*-deficient mice also demonstrate blunted LPS-induced septic shock compared with wild-type mice (Liu et al. 2006), indicating a

functional change that has important biological implications. These studies demonstrate the significant disturbances that can occur as a consequence of a disrupted molecular circadian clock.

In addition to genetically manipulating circadian homeostasis, environmentally disrupting circadian rhythms also negatively affects immune function. For example, loss of regular sleep/wake cycles alters the normal circadian rhythmicity observed in immune cells (Bryant et al. 2004; Vgontzas et al. 2004) and increases the susceptibility to infections (Everson 1993; Mohren et al. 2002). Indeed, chronically shifting light/dark cycles in mice augments LPS-induced immune response, resulting in greater mortality compared with non–circadian-disrupted mice (Castanon-Cervantes et al. 2010).

Taken together, these studies provide evidence that circadian disruption can significantly, and typically negatively, influence immune function. Therefore, alcohol-induced circadian disruption may be a susceptibility factor for immune dysregulation, which may promote alcohol-associated inflammatory processes. Furthermore, the altered response to LPS has particular relevance in light of the alcohol-induced effects on intestinal permeability.

Circadian Rhythms and Metabolic Syndrome

Although only a few metabolic genes are direct targets of circadian genes (Noshiro et al. 2007; Panda et al. 2002), the direct targets do include many transcription factors and other modulators of transcription and translation. These clock-controlled genes include factors regulating lipid and cholesterol biosynthesis, carbohydrate metabolism, oxidative phosphorylation, and glucose levels (Oishi et al. 2003; Panda et al. 2002).

Eating is an environmental factor that selectively affects peripheral circadian rhythmicity in the intestine and liver. Feeding at the incorrect time (e.g., late-night eating for humans) can result in internal circadian misalignment. For example, restricted feeding paradigms in which animals only have access to food during inappropriate times (i.e., during the light cycle for nocturnal rodents) results in misalignment between central light-entrained circadian rhythms (i.e., in the SCN) and peripheral food-entrained circadian rhythms, including those in the liver (Damiola et al. 2000). Recent studies suggest that this internal misalignment scenario is linked to weight gain, obesity, and metabolic syndrome. Indeed, mice fed during the inappropriate time gain more weight (Arble et al. 2009; Salgado-Delgado et al. 2010) than mice fed during appropriate time, despite similar activity levels and caloric intake (Arble et al. 2009). This phenomenon also is observed in humans; people who skip breakfast and have eating patterns shifted toward late-night eating tend to be more overweight than those who consume food during more appropriate time periods (Berkey et al. 2003; Ma et al. 2003).

Feeding at the incorrect time (e.g., late-night eating for humans) can result in internal circadian misalignment.

Genetic abnormalities in the molecular circadian clock also are associated with metabolic disorders, including obesity, metabolic syndrome, and diabetes (Scott et al. 2008; Woon et al. 2007). For example, *Clock* mutant mice, which have disrupted circadian rhythms (Vitaterna et al. 1994), are obese and demonstrate characteristics of metabolic syndrome such as high cholesterol levels and high blood glucose (Turek et al. 2005). *Bmal1* mutant mice also have disrupted circadian rhythmicity (Bunger et al. 2000), disrupted adipogenesis (Shimba et al. 2005), and demonstrate markers of metabolic syndrome (e.g., higher levels of triglycerides and glucose) (Marcheva et al. 2010; Rudic et al. 2004). Similarly, mutations in *Cry* genes disrupt hormonal rhythms (Fu et al. 2005; Yang et al. 2009) and *Cry* mutants show markers of metabolic syndrome (Okano et al. 2009). It should be noted that although some of these mutant mice demonstrate disrupted locomotion and feeding behaviors (i.e., wrong-time feeding), the abnormalities seem to be attributable to mutations in the circadian clock machinery rather than to appropriate feeding times because mice (e.g., *Bmal1* mutant mice) that do exhibit normal activity/feeding patterns still exhibit markers of metabolic syndrome (Lamia et al. 2008; Marcheva et al. 2010).

In addition to these effects of circadian rhythms on indices of metabolism, it is also important to consider the effect of circadian disruption on the immune system because chronic inflammation is a prominent feature associated with metabolic syndrome. Thus, the immune dysfunction that occurs upon circadian rhythm disruption may be a predisposing or exacerbating factor for metabolic syndrome.

Epigenetic Alterations: Circadian Rhythm Disruption and Alcohol

Epigenetics is the study of stable changes in gene expression that do not involve DNA sequence modifications but rather are the consequence of processes such as DNA methylation, histone modification (i.e., acetylation, methylation, phosphorylation, ubiquitinylation, ADP-ribosylation, and sumoylation), and noncoding microRNAs (miRNAs). These changes in gene expression are critical to optimize cellular function and for cellular development and differentiation. However, epigenetic changes also occur in response to environmental changes,

including circadian rhythm disruption and alcohol use.

Shift work (i.e., chronic circadian disruption) is associated with an increased incidence of cancer. Potential mechanisms for this relationship include changes in melatonin levels and levels of circadian clock genes (Straif et al. 2007). However, epigenetics also may influence circadian rhythm disruption and thereby affect cellular function. Indeed, long-term shift work affects promoter methylation of the circadian genes *Clock* and *Cry2* (Zhu et al. 2011) with increased methylation of *Clock* (Hoffman et al. 2010*a*) and decreased methylation of *Cry* (Hoffman et al. 2010*b*) observed in cancer patients. Epigenetic changes also occur as a consequence of chronic circadian disruption in the promoter regions of genes encoding glucocorticoid receptors (important for hypothalamic–pituitary–adrenal axis function), TNFα (a cytokine critical for cell functioning and inflammation), and IFNγ (Bollati et al. 2010). Changes such as these may play a critical role in how chronic circadian disruption promotes cancer, inflammation, and metabolic disorders.

In addition to circadian-disruption–induced epigenetic changes, alcohol consumption is also associated with epigenetic modifications. Alcohol-induced DNA acetylation is observed in vitro in rat hepatocytes (Park et al. 2003), in vivo in rat hepatic stellate cells (Kim and Shukla 2005, 2006), lung, spleen, and testes (Kim and Shukla 2006). Similar to the increased cancer risk associated with chronic circadian disruption, alcohol-induced epigenetic changes are associated with the development of cancer. Indeed, colorectal cancer in high-alcohol– consuming humans is associated with high levels of promoter hypermethylation of several relevant genes when compared with low- or no-alcohol– consuming counterparts with colorectal cancer (van Engeland et al. 2003; Giovannucci et al. 1995). Similarly, alcohol-consuming individuals with head and neck cancer have hypermethylated gene promoters for specific genes of interest compared with non-alcohol–drinking individuals (Puri et al. 2005) and alcohol-dependent humans have hypermethylation of liver and peripheral blood cell DNA. Thus, it seems that both circadian disruption and alcohol consumption can affect long-term changes in gene expression via epigenetic modifications that may impact a wide variety of health outcomes.

Summary and Future Directions

Circadian rhythms are a prominent and critical feature of cells, tissues, organs, and behavior that help an organism function most efficiently and anticipate things such as food availability. Therefore, it is not surprising that disrupted circadian rhythms or misalignment between central and peripheral circadian rhythms predispose and/or exacerbate a wide variety of diseases, including alcohol-associated disorders. One environmental factor that has been shown to have a disruptive effect on circadian rhythms is alcohol consumption. This disruption occurs at the molecular levels (i.e., changes in the expression levels of the circadian clock genes), also affects tissues and organs (e.g., changes in the cyclic pattern of hormones), and leads to overt behavioral changes. Thus, in the context of alcoholism, disrupted circadian rhythms may create a positive feedback loop that markedly exaggerates alcohol-induced immune/inflammatory-mediated diseases by (1) negatively influencing immune function and (2) promoting alcohol consumption that leads to further circadian-rhythm disruption. These changes are highly relevant because circadian-rhythm disruption has a substantial impact on immune function, which in turn has important implications for a wide variety of pathological conditions, including metabolic syndrome. A better understanding of how circadian rhythms influence such a wide variety of systems and bodily functions and how environmental factors such as alcohol use influence these processes is vital to our ever more circadian-disrupted society.

A better understanding of the mechanisms by which circadian disruption affects health outcomes such as cancer, inflammation, metabolic disease, and alcohol-induced pathology is critical. This information may lead to the development of chronotherapeutic approaches to prevent and/or treat a wide variety of conditions that are promoted or exacerbated by circadian-rhythm disruption and may lead to better risk stratification for individuals who are at risk for developing chronic conditions. Going forward, characterizing the epigenetic modifications that occur during chronic circadian disruption may be critical for understanding not only how disruption affects an individual but also how these modifications are passed on to offspring, which may influence the health of future generations. Thus, the issue of circadian disruption is vitally important for the health and well-being of current and future generations. ■

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Epigenetic Control of Gene Expression in the Alcoholic Brain

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Chronic alcohol exposure causes widespread changes in brain gene expression in humans and animal models. Many of these contribute to cellular adaptations that ultimately lead to behavioral tolerance and alcohol dependence. There is an emerging appreciation for the role of epigenetic processes in alcohol-induced changes in brain gene expression and behavior. For example, chronic alcohol exposure produces changes in DNA and histone methylation, histone acetylation, and microRNA expression that affect expression of multiple genes in various types of brain cells (i.e., neurons and glia) and contribute to brain pathology and brain plasticity associated with alcohol abuse and dependence. Drugs targeting the epigenetic "master regulators" are emerging as potential therapeutics for neurodegenerative disorders and drug addiction. **KEY WORDS: Alcohol consumption; alcoholism; chronic alcohol exposure; alcohol use, abuse and dependence; epigenetics; epigenetic therapeutics; gene expression; brain; brain cells; brain pathology; behavior; DNA methylation; histone; microRNA; transcription; pharmacotherapy; animal models; human studies**

Whether a specific gene is transcribed or repressed is determined by the specific status (i.e., conformational state) of the complex of chromosomal DNA and proteins (i.e., the chromatin) and by the recruitment of specific proteins (i.e., transcription factors) to regulatory sites on the DNA (Copeland et al. 2010). Chromatin states can change as a result of enzyme-mediated covalent modifications of the DNA and structural chromatin proteins (i.e., histones) (Borrelli et al. 2008; Copeland et al. 2010). These changes in chromatin, which are often termed epigenetic marks, include such modifications as DNA methylation and histone methylation and acetylation. In addition, incorporation of histone variants, adenosine triphosphate (ATP)-dependent chromatin remodeling, and regulation of gene expression by noncoding RNAs also are considered epigenetic phenomena and play important roles in regulation of gene expression. It is becoming increasingly clear that epigenetic mechanisms play a key role in cellular differentiation and regulation of cell type–specific transcriptional programs, producing a remarkable heterogeneity of cellular transcriptomes[1] that reflect the physiological properties and functional state of individual cells.

The brain arguably is one of the most complex biological tissues and enables the organism to sense, remember, and respond to its environment. It constantly adapts to environmental stimuli through regulated changes in gene expression. Chronic alcohol exposure causes widespread changes in brain gene expression in humans and animal models (Mulligan et al. 2006; Ponomarev et al. 2012), and there is evidence that many of these changes mediate the processes of cellular adaptation leading to addiction (Mayfield et al. 2008). Until recently, the role of epigenetic processes in alcohol's effects on the central nervous system (CNS) has been largely understudied. However, in the past 5 years the number of studies that suggested a role for epigenetics in alcohol-related molecular and behavioral changes has grown considerably. This review summarizes evidence for the role of epigenetic modifications in alcohol's effects on brain gene expression and behavior.

[1] The first step of gene expression (i.e., transcription) involves the synthesis of intermediary molecules called messenger RNAs (mRNAs) that are copies of the gene(s) to be expressed and which serve as templates for the synthesis of the encoded protein(s) during the second step of gene expression (i.e., translation). A transcriptome is the entirety of all mRNAs found in a certain cell, organ, or organism.

DNA Methylation

DNA methylation generally is associated with transcriptional repression. It mainly occurs at sites where a cytosine and a guanosine nucleotide are located next to each other (i.e., CpG dinucleotides). If these CpG dinucleotides are located within regulatory sequences, such as promoter regions, their methylation can block the binding of transcription factors and/or establish a repressive chromatin state (Renthal

and Nestler 2009*b*). One of the first indications that DNA methylation may play a role in alcoholism can be traced back to 1940s and 1950s, to the work of Dr. Roger J. Williams, a biochemistry professor at the University of Texas at Austin. He showed for the first time that dietary changes could affect beverage alcohol (i.e., ethanol) consumption in rodents. Specifically, diets deficient in B vitamins (e.g., folic acid and choline) increased consumption of solutions containing 10 percent ethanol in some rats, whereas vitamin-enriched diets decreased it (Williams et al. 1949). It now is well established that folates and several other B vitamins are critical for one-carbon metabolism and the synthesis of a compound called *S*-adenosyl-methionine (SAM), which serves as the primary methyl group donor in most transmethylation reactions, including DNA methylation (Hamid et al. 2009). Therefore, it is possible that dietary changes in this early study affected alcohol consumption via changes in DNA methylation and methylation-regulated gene expression.

Chronic alcohol consumption causes well-documented vitamin B and folate deficiencies that negatively affect the biochemical reactions in which a chemical unit containing one carbon atom (e.g., a methyl group) is transferred through several steps from a donor to another compound, such as DNA (i.e., one-carbon metabolism). These effects on one-carbon metabolism can result in excess levels of the SAM precursor homocysteine in the blood (i.e., homocysteinemia) and decreased SAM production (Blasco et al. 2005; Hamid et al. 2009). In addition, alcohol can affect DNA methylation through several other mechanisms, including the following:

- The alcohol metabolite, acetaldehyde, may induce inhibition of an enzyme called DNA methyltransferase 1 (DNMT1) that mediates most DNA methylation reactions needed to maintain the cell's normal functioning (Garro et al. 1991).
- Alcohol-induced DNA damage and the resulting repair reactions can lead to demethylation of 5-methylcytosine nucleotides (Chen et al. 2011).

Both of these mechanisms can cause reduced levels of methylation throughout the DNA (i.e., global DNA hypomethylation), a chromatin state associated with many pathological conditions, including cancer (Pogribny and Rusyn 2012). Alcohol-induced global DNA hypomethylation has been reported in several peripheral tissues of alcohol-related models and may play a role in alcoholic liver disease, fetal alcohol syndrome, and colon cancer (Choi et al. 1999; Garro et al. 1991; Hamid et al. 2009; Lu et al. 2000; Shukla et al. 2008). However, the effect of chronic alcohol on global DNA methylation seems to be tissue specific because one study reported enhanced DNA methylation (i.e., global DNA hypermethylation) in a certain type of blood cells (i.e., peripheral mononuclear cells) in alcoholic patients undergoing early alcohol withdrawal (Bonsch et al. 2004).

Two recent studies (Manzardo et al. 2012; Ponomarev et al. 2012) have examined alcohol's effects on global DNA methylation in the brain. Both studies measured DNA methylation in the frontal cortex of chronic alcoholics and matched control cases, but using two different methods. Ponomarev and colleagues (2012) studied genomic regions that included DNA sequences called long terminal repeat (LTR)-containing retrotransposons, also known as endogenous retroviruses (ERVs), most of which are nonfunctional remnants of ancient retroviral infections (Antony et al. 2004). The investigators showed that these repeats, which usually are heavily methylated, were less methylated in alcoholic brains, which was associated with their increased expression. Because ERVs constitute a significant part of the human genome, the study concluded that alcohol abuse causes global DNA hypomethylation in the brain, which is consistent with the majority of previous studies on alcohol-induced changes in DNA methylation. Manzardo and colleagues (2012) used immunological methods (i.e., immunoprecipitation) to isolate methylated DNA from alcoholics and control subjects and then applied this DNA to microarrays containing genomic promoter regions to identify promoters for which the methylation patterns differed between the two groups. The analyses found no differences between the groups in total methylation at the whole-genome level; however, about 20 percent of all promoters were differentially methylated between the groups, with less than half of these promoters showing greater methylation in alcoholics.

These complementary findings suggest that chronic alcohol causes a general decrease in the overall number of methylated cytosines but also could lead to the de novo methylation of previously unmethylated nucleotides at the promoters of some genes. Such a combination of these processes already has been widely reported in studies of cancer, showing, for example, that methyl-deficient diets induce development of liver tumors (i.e., hepatocarcinogenesis) associated with global DNA hypomethylation and promoter hypermethylation at specific genes (Ehrlich 2005; Pogribny and Rusyn 2012). Hypomethylated states associated with cancer and other pathological conditions often are accompanied by a downregulation of the gene encoding DNMT1 (Hervouet et al. 2010), which also has been observed in the brains of chronic alcoholics (Ponomarev et al. 2012). These striking similarities point to some common mechanisms of methyl deficiency across tissues.

Studies assessing epigenetic regulation of individual genes in the brain have shown that alcohol's effects on DNA methylation depend on a variety of factors, including the specific gene targets, developmental stage of exposure, and type of neuronal tissue affected. Much of this work has focused on the central effects of prenatal alcohol exposure and on gene regulation in cell cultures. Prenatal exposure of rats

to alcohol resulted in DNA hypermethylation and a reduced expression of a protein called brain-derived neurotrophic factor (BDNF) in olfactory bulbs of rat pups, which was associated with loss of neurons in this brain region (Maier et al. 1999). Similar molecular results were obtained in a separate study where prenatal alcohol treatment of rats led to DNA hypermethylation and a decreased expression of a protein characteristically found in brain cells called astrocytes (i.e., glial fibrillary acidic protein [GFAP]) in the brains of the pups (Valles et al. 1997). In neural cell cultures, alcohol-induced downregulation of cell-cycle genes was paralleled by an increased DNMT activity and hypermethylation of the promoters of those genes (Hicks et al. 2010). Conversely, upregulation of the gene encoding a receptor subunit for the neurotransmitter glutamate (i.e., the NMDA NR2B receptor subunit) was associated with demethylation of CpG dinucleotides in the gene's promoter after chronic alcohol (Marutha Ravindran and Ticku 2004).

However, some reports suggest that the relationship between DNA methylation and the expression of neighboring genes may be even more complex than previously thought (Ehrlich 2005). For example, a recent study demonstrated an increased expression of a signaling molecule called prodynorphin (PDYN) that was associated with methylation of a CpG dinucleotide located in a DNA region behind the actual protein-coding region of the gene (i.e., in the 3′-untranslated region of the gene) in the brains of alcohol-dependent people (Taqi et al. 2011), although no causal link was established.

Specific DNA methylation patterns differ among tissues and cell types, and these differences contribute to establishing the cells' epigenetic landscape and transcriptional programs and defining cellular identity (Bernstein et al. 2007). Also, although alcohol's general effects on DNA methylation may be similar across various tissues, the specific genes affected by this regulation may differ depending on cell type. The epigenetic regulation of such proteins as GFAP, which is a marker of astrocytes, and the NR2B subunit, which generally is expressed in neurons, suggests that alcohol-induced epigenetic changes will affect molecular markers of individual cell types to a greater degree than other proteins. Many studies of alcohol's epigenetic modification of the chromatin have been conducted in blood cells obtained from alcoholics (Biermann et al. 2009; Bonsch et al. 2005; Hillemacher et al. 2009). Because of the concern regarding the tissue specificity of alcohol's epigenetic effects, however, the results of these important studies cannot be readily generalized to mechanisms in brain. Therefore, parallel measurements of the entirety of all alcohol-induced epigenetic changes (i.e., the epigenomic changes) in the blood and brain should be obtained and vigorously compared in animal models to detect common patterns, based on which generalization of results in humans can be made.

Histone Modifications

Histone proteins are the second major target of epigenetic changes. These proteins can be modified by a relatively large number of specific enzymes that mediate covalent attachment and removal of four classes of chemical groups: methyl, acetyl, phosphate, and ubiquitin (Bernstein et al. 2007; Borrelli et al. 2008). Studies of alcohol-induced modifications mainly have focused on two histone modifications: a trimethylation of histone 3 at the lysine 4 residue (H3K4me3), which is a promoter-enriched chromatin mark of actively transcribed genes, and acetylation of various residues of histones 3 and 4 (H3 and H4). Histone acetylation generally is associated with a more open, accessible structure of the chromatin and, consequently, increased transcription, whereas deacetylated histones can cause transcriptional repression (Bernstein et al. 2007).

Chronic alcohol abuse in humans can result in global and gene-specific increases in H3K4me3 in the brain cortex (Ponomarev et al. 2012) and in either increases or decreases of this modification in promoters of specific genes in the hippocampus (Zhou et al. 2011). The latter study used a combination of two techniques (i.e., chromatin immunoprecipitation followed by DNA sequencing [ChIP-Seq]) to detect individual genes with differences between alcoholics and control subjects in H3K4 promoter trimethylation and in parallel measured the levels of transcription of the same genes. Interestingly, differences in promoter methylation did not correlate with differences in gene expression, suggesting that H3K4me3 status alone is not a reliable predictor of genome-wide steady-state mRNA levels at a given time point. A possible explanation of these results is that the H3K4me3 mark in the promoter regions only indicates that the chromatin is in an open conformation that is accessible to regulatory or transcription factors but does not mean that transcription actually is initiated and the transcription machinery is present (Bernstein et al. 2007). A recent study (D'Addario et al. 2011) supports this hypothesis as well as previous findings showing mechanistically linked but temporally complex relationships between chromatin marks at gene promoters and mRNA abundance. The investigators explored the effects of ethanol and its metabolite acetaldehyde on various chromatin marks and the transcription of the *PDYN* gene in a human cell line derived from a tumor arising from nerve tissue cells (i.e., a neuroblastoma). The analyses suggested that the ethanol-induced increase in H3K4me3 that was observed after 72 hours of ethanol exposure did not result in initiation of *PDYN* transcription but kept the gene in a poised state for later reactivation. This is consistent with other findings regarding *PDYN* activation in human alcoholics (Taqi et al. 2011).

Most evidence to date on the role of central epigenetic processes in alcoholism has been collected from studies focusing on histone acetylation, often by

modifying the activities of the enzymes that add acetyl groups (i.e., histone acetyl transferases [HATs]) or remove acetyl groups (i.e., histone deacetylases [HDACs]). Particularly, small molecules that inhibit HDAC function (HDACis) and thus result in increased histone acetylation have been investigated intensely in recent years. These molecules are attractive because they can enter the brain via the blood (i.e., cross the blood–brain barrier) and exert a broad range of effects in the CNS, including enhanced memory formation as well as anti-inflammatory and neuroprotective effects (Kazantsev and Thompson 2008; Sweatt 2009). Several studies using HDACis demonstrated effects of altered histone acetylation on different alcohol-related behaviors, including withdrawal-related anxiety (Pandey et al. 2008), locomotor sensitization (Sanchis-Segura et al. 2009), alcohol consumption (Wostenholme et al. 2011), conditioned place aversion (Pascual et al. 2012), and rapid tolerance (Sakharkar et al. 2012). For example, Pandey and colleagues (2008) showed that acute ethanol increased H3K9 and H4K8 acetylation in rats, whereas anxiety-like behaviors during withdrawal after chronic alcohol exposure were associated with decreases in these acetylation marks, decreased expression of several proteins (e.g., CREB-binding protein [CBP] and neuropeptide Y [NPY]), and increased HDAC activity. However, treatment with the HDACi, trichostatin A (TSA), to block HDAC activation prevented the deficits in gene expression and the development of withdrawal-related anxiety. Sanchis-Segura and colleagues (2009) demonstrated that treatment of mice with another HDACi (i.e., sodium butyrate) altered some alcohol-related behaviors (e.g., enhanced ethanol-induced locomotor sensitization) but had no effect on others (e.g., ethanol tolerance or withdrawal). Finally, daily injections of TSA in mice that had continuous access to both water and an alcohol solution increased the animals' alcohol consumption (Wolstenholme et al. 2011).

Similar to DNA methylation, alcohol's effects on histone acetylation are tissue, brain region–, and cell type–specific. For example, a single dose of ethanol[2] into the stomach increased the levels of H3 acetylation in the liver, lungs, and testes but had no effects in other tissues, including whole brain, of rats (Kim and Shukla 2006). In the brain, ethanol-induced changes in H3/H4 acetylation were observed in the central and medial but not the basolateral nuclei of the amygdala (Pandey et al. 2008; Sakharkar et al. 2012); moreover, the increased histone acetylation appeared to be specific for neurons (Sakharkar et al. 2012).

Other factors that can affect alcohol-induced changes in histone acetylation include species, the organism's specific genetic makeup (i.e., genotype), age, the dose and route of ethanol administration, and duration of exposure. For example, ddY mice treated with chronic ethanol vapor showed increases of both global and gene-specific histone acetylation in the ventral midbrain during withdrawal that peaked around 10 hours post ethanol (Shibasaki et al. 2011). Also, intermittent alcohol exposure produced different effects on histone acetylation in adolescent and adult rats, with juvenile animals generally showing more changes (Pascual et al. 2009, 2012). Consistent with these studies was the finding that ethanol exposure during the early postnatal period in rats resulted in a marked reduction of CBP levels and histone acetylation in the developing cerebellum (Guo et al. 2011). In addition, possible interactions among various factors may result in different time courses for alcohol-induced changes, because histone acetylation measured 24 hours after the last of repeated alcohol injections was increased in some brain areas (e.g., frontal cortex and nucleus accumbens), decreased in others (e.g., striatum), and unchanged in still others (e.g., hippocampus) (Pascual et al. 2009).

Histone acetylation generally is associated with transcriptional activation, but similar to the H3K4me3 mark, the relationships between levels of histone acetylation and steady-state mRNA are complex, because activation of different genes is associated with acetylation of different residues of H3 and H4 at different time points (Renthal and Nestler 2009*a*). And although alcohol's effects on histone acetylation now are well established, the exact mechanisms underlying this influence on gene expression are not well understood. Alcohol-induced changes in histone acetylation are paralleled by regulation of several genes, including *CBP, NPY* (Pandey et al. 2008), *FosB* (Pascual et al. 2012), and *NR2B* (Qiang et al. 2011). One proposed mechanism involves the transcription factor CREB, to which CBP can bind (Moonat et al. 2010). CBP has intrinsic HAT activity and, when recruited by CREB, can promote transcriptional activation by acetylating histones. This mechanism has been shown to play a role in cocaine-induced regulation of *FosB* (Levine et al. 2005). A similar mechanism also was proposed to regulate H4 acetylation, transcription of the gene encoding the BK-type potassium channel, and tolerance to benzyl alcohol in the fruit fly, Drosophila (Wang et al. 2007).

Gene expression experiments have provided additional support for the role of histone acetylation in alcohol addiction. Several studies focusing on brain changes in human alcoholics have shown general downregulation of genes involved in histone acetylation and upregulation of genes promoting histone deacetylation. The latter group of genes includes those encoding proteins forming so-called transcription corepressor complexes (TCCs), which help suppress transcription by coupling HDAC activity with DNA methylation, thereby establishing a repressive chromatin state (McDonel et al. 2009). For example, transcripts of the genes encoding CREB and CBP were downregulated in alcoholics (Ponomarev et al. 2012). Conversely, transcripts of the gene *MBD3,* which encodes a key player in TCCs called methyl-CpG–binding protein, as well as many other TCC genes, such as *SIN3A, SIN3B,*

[2] The dose administered was 6 g/kg of a 32 percent ethanol solution.

player in TCCs called methyl-CpG–binding protein, as well as many other TCC genes, such as *SIN3A, SIN3B, MTA1, MTA2, RBBP4, GATAD2A, GATAD2B,* and *CHD4* were upregulated in alcoholics (Liu et al. 2006; Ponomarev et al. 2012; Zhou et al. 2011). Together, these observations validate previous findings that histone acetylation is decreased during alcohol withdrawal (Pandey et al. 2008) and suggest that TCCs are activated and play a role in the downregulation of some genes in the alcoholic brain.

MicroRNAs

MicroRNAs (miRNAs) comprise a specific class of noncoding RNAs that bind to complementary sequences on target mRNAs to repress translation and silence gene expression (Robison and Nestler 2011). Expression of miRNAs can alter the transcriptional potential of a gene in the absence of any change to the DNA sequence and therefore can be considered an epigenetic phenomenon. The most convincing evidence for the involvement of miRNAs in alcohol-related gene expression was presented by Pietrzykowski and colleagues (2008), who showed that alcohol upregulates expression of microRNA 9 (miR-9) in rat brain, which results in miR-9–dependent downregulation of BK channel variants with high sensitivity to alcohol. This mechanism is proposed to mediate the development of cellular tolerance and generally may contribute to neuronal adaptation to alcohol.

Additional evidence for the role of miRNAs in alcohol-induced regulation of gene expression and behavior comes from genomic studies measuring levels of multiple miRNAs after exposure to alcohol. Using neural cultures and a model of alcohol-induced teratogenesis, Sathyan and colleagues (2007) identified the first alcohol-sensitive miRNAs. Subsequent studies using miRNA microarrays detected multiple alcohol-regulated miRNAs in neural cultures (Yadav et al. 2011), fetal mouse brains (Wang et al. 2009), and brains of human alcoholics (Lewohl et al. 2011).

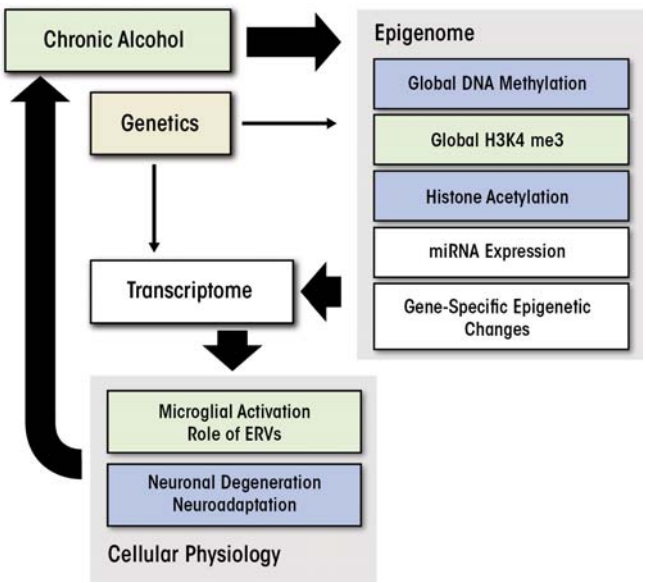

Figure A hypothetical diagram for the role of epigenetic modifications in alcohol addiction. Yellow color indicates general increase, up-regulation, or activation, whereas blue color indicates general decrease, down-regulation, or degeneration. White background implies bidirectional changes. Potential interactions between different components of the diagram are discussed in the text.

Summary and Future Directions

The findings reviewed in this article point to a central role of various epigenetic processes in controlling alcohol-induced changes in brain gene expression and behavior, which may play an important part in the development of alcohol addiction (see the figure). For example, chronic alcohol exposure can result in global DNA hypomethylation via several mechanisms, including vitamin B and folate deficiencies that can lead to an impairment of one-carbon metabolism and a decrease in SAM levels. However, these global effects of alcohol do not imply unidirectional changes across the whole genome, because many genes show the opposite epigenetic changes in their promoters.

Many of the observed chromatin modifications are mechanistically linked, resulting in a limited number of chromatin states (Jaenisch and Bird 2003). For example, trimethylation of H3K4 is mechanistically coupled with unmethylated DNA (Hashimoto et al. 2010), suggesting that the reduced DNA methylation observed in alcoholic brains can promote a general increase in the H3K4me3 levels. Histone actetylation patterns also are commonly altered by alcohol in a process that may be linked to DNA methylation. Thus, acute alcohol exposure promotes histone acetylation, whereas withdrawal from chronic alcohol often increases deacetylation of histones. Deacetylation via HDAC activity is coupled to DNA methylation through the actions of methyl-binding proteins and other TCC components. Chronic alcohol exposure leads to upregulation of TCC genes, which may serve to compensate for the reduced number of methylated CpGs. These cumulative changes in

ferent cell types and lead to activation of microglia, neuronal degeneration, and compensatory neuroadaptations in alcoholic brain. In summary, alcohol-induced epigenetically mediated changes in gene expression may underlie the brain pathology and adaptations in brain functioning (i.e., brain plasticity) associated with alcohol abuse and alcohol dependence and may contribute to alcohol relapse and craving.

To advance the current state of epigenetic research in alcoholism, future studies that look at both simplified models and entire regulatory systems (i.e., that use both reductionist and systems approaches) are needed. One focus of this research should be on understanding the exact mechanistic links between chronic alcohol exposure, epigenetic changes, and gene expression. Exploratory studies likely will first use discovery-driven approaches to investigate the mechanistic relationships between the epigenome and the transcriptome in animal models and formulate hypotheses at the single-gene, gene-network, and systems levels. Follow-up studies using both animal models and human postmortem material then can help test these hypotheses and validate functional predictions of the genome-wide experiments. Because of the complex temporal relationships between chromatin marks and transcriptional changes, time-course studies also will be required. The recently available epigenetic maps from the ENCODE (ENCyclopedia Of DNA Elements) Project (Dunham et al. 2012) should help accelerate these research efforts.

To address the causal relationships between epigenetic modifications and alcohol traits, it will be essential to use tools of both forward and reverse genetics. Forward-genetics approaches seek to determine the genetic basis of an observed trait (i.e., phenotype). Such approaches include mapping DNA regions that may contain disease-related genes (i.e., quantitative trait loci [QTLs]), using chromatin modifications as phenotypes. This can be achieved using genetic reference panels, such as recombinant inbred strains of mice and rats (Rosen et al. 2007). Many reference populations have been tested extensively for both expression of specific genes and alcohol-related behaviors and therefore can serve as powerful tools for integrating data across biological modalities and investigating mechanistic links between the genome and the entirety of all analyzed phenotypes (i.e., the phenome) through genetic mapping of the epigenome and the transcriptome. Conversely, reverse-genetics approaches study the phenotypes that arise as the result of alterations of particular genes. An example of a reverse-genetics approach is to assess alcohol-related behaviors in mice with genetic mutations of chromatin-binding proteins.

Another important research direction is to investigate the cellular specificity of alcohol-induced epigenetic changes. For example, future research should determine cell type–specific chromatin states that drive the unique molecular responses to alcohol in different neurons and glial cells and show how epigenetic modifications help establish functional states consistent with the pathophysiological changes observed in alcoholism. One example of this approach is the analysis of the role of epigenetically controlled ERVs in alcohol addiction (Ponomarev et al. 2012). Previous studies found that an ERV-encoded glycoprotein called syncytin can directly activate different types of glial cells (i.e., microglia and astrocytes) and induce neuroinflammation (Antony et al. 2004). Microglial activation, in turn, can result in neuronal degeneration (Crews et al. 2011), and syncytin-activated astrocytes can secrete compounds that are toxic to other glial cells (i.e., oligodendrocytes) and thus lead to myelin degeneration (Antony et al. 2004). Both of these effects are consistent with pathologies observed in alcoholics (Harper et al. 2003; Pfefferbaum et al. 2009; Zahr et al. 2011). Alcohol-induced neuroimmune responses have been suggested to be a critical factor in alcohol addiction (Crews et al. 2011), and Ponomarev and colleagues (2012) proposed a novel mechanism including the potential role for ERVs in neuroinflammation and brain pathophysiology of human alcoholism. Another approach to assessing the cell specificity of epigenetic processes is to compare alcohol-induced epigenetic changes across tissues and cell types. Human research often is limited to peripheral tissues (e.g., blood). To be able to draw parallels between peripheral and central mechanisms in humans, researchers first need to study the relationships between responses to alcohol in the brain and those in other tissues using animal models.

Other research efforts should focus on the potential exploitation of epigenetic mechanisms for alcoholism treatment. Epigenetic therapeutics, such as HDACis, offer unique advantages in treating diseases through chromatin-dependent changes in gene expression. These "master regulators" can affect expression of multiple genes. Therefore, in order to understand the effects of these agents on alcohol behaviors, it is important to study their mechanisms of action and identify the range of genes and molecular pathways affected. Large-scale genomic studies should focus on the global relationships between chromatin marks and gene expression in the context of chronic alcohol exposure and epigenetic therapeutics. Finally, multiple studies in humans and animal models have highlighted the importance of the genetic component in alcohol addiction (Crabbe 2008; Mayfield et al. 2008; Spanagel 2009). To better understand the interplay between genetic, epigenetic, and environmental factors in controlling gene expression in alcoholism, integrative approaches across studies are warranted. Many epigenetic therapeutics have been developed for other diseases, and understanding the functional relationships between epigenetic processes and the transcriptome in the alcoholic brain may lead to new molecular targets for medication development for human alcoholism. ■

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Resilience to Meet the Challenge of Addiction

Psychobiology and Clinical Considerations

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Acute and chronic stress–related mechanisms play an important role in the development of addiction and its chronic, relapsing nature. Multisystem adaptations in brain, body, behavioral, and social function may contribute to a dysregulated physiological state that is maintained beyond the homeostatic range. In addition, chronic abuse of substances leads to an altered set point across multiple systems. Resilience can be defined as the absence of psychopathology despite exposure to high stress and reflects a person's ability to cope successfully in the face of adversity, demonstrating adaptive psychological and physiological stress responses. The study of resilience can be approached by examining interindividual stress responsibility at multiple phenotypic levels, ranging from psychological differences in the way people cope with stress to differences in neurochemical or neural circuitry function. The ultimate goal of such research is the development of strategies and interventions to enhance resilience and coping in the face of stress and prevent the onset of addiction problems or relapse. **KEY WORDS: Addiction; substance abuse; stress; acute stress reaction; chronic stress reaction; biological adaptation to stress; psychological response to stress; physiological response to stress; resilience; relapse; coping skills; psychobiology**

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Evidence from different disciplines suggests that acute and chronic stress–related mechanisms play an important role in both the development and the chronic, relapsing nature of addiction (Baumeister 2003; Baumeister et al. 1994; Brady and Sinha 2005). Stress is defined as the physiological and psychological process resulting from a challenge to homeostasis by any real or perceived demand on the body (Lazarus and Fokman 1984; McEwen 2000; Selye 1976). Stress often induces multisystem adaptations that occur in the brain and body and affect behavioral and social function. The resulting dynamic condition is a dysregulated physiological state maintained beyond the homeostatic range. This definition and conceptualization of stress was further developed to explain the chronic abuse of substances and comfort foods and has been studied in the context of behavioral addiction (i.e., pathological gambling) (Dallman et al. 2005; Koob and Le Moal 1997; Koob 2003).

Persistent challenges to an organism through chronic substance use may ultimately lead to an altered set point across multiple systems. This hypothesis is consistent with evidence that suggests adaptations in brain reward and stress circuits, and local physiology (e.g., energy balance) can contribute to addictive processes. Cravings or urges, decreases in self-control, and a compulsive engagement in unhealthy behaviors each characterize patients with addiction (Dallman et al. 2005; Kalivas and

Volkow 2005; Koob et al. 2004; Sinha 2001). Alternatively, a person's ability to successfully cope with high stress is reflected in adaptive physiological and psychological responses (Charney 2004; MacQueen et al. 2003).

Resilience, defined as the absence of psychopathology despite exposure to high stress, can be studied by examining interindividual differences in stress responsivity across an organism's various types (i.e., at multiple phenotypic levels). Responsivity ranges from psychological differences in the way individuals cope with stress to differences in neurochemical or neural circuitry function (Cicchetti and Blender 2006). Variability within the genetic makeup and quality of early-life experience, as well as interactions between the two, are known to contribute to differences in stress resilience (Enoch 2010; Heim and Nemeroff 2001). Genetic influences can stem from gene–environment interactions, changes in gene expression influenced by the environment (i.e., epigenetic changes), or variation within the actual genetic code. Some examples of genetic influences on resilience include variability in the genes involved in the body's stress response (i.e., those controlling the hypothalamic–pituitary–adrenal [HPA] axis). These include those coding for the corticotropin-releasing factor (CRF) type 1 receptor or the glucocorticoid receptor (GR) (which cortisol can activate) as well as the serotonin transporter cathecol-O-methyltransferase (COMT), neuropeptide Y (NPY), and brain-derived neurotrophic factor (BDNF) genes (Feder et al. 2009) Genetic variation in the gene encoding the CRH1 receptor was found to moderate the impact of stress, for example, among adolescents engaging in heavy drinking (Blomeyer et al. 2008; Schmid et al. 2010). This gene-by-environment interaction predicted the initiation of drinking in adolescence as well as progression to heavy drinking by young adulthood (Schmid et al. 2010). The following sections highlight resilient responses to stress in studies in which stress was identified as an important factor contributing to the neurobiology of alcohol dependence.

Psychosocial Factors Associated With Resilience

Early studies of children exposed to adversity (Masten 2001; Masten and Coatsworth 1998; Rutter 1985) as well as more recent studies in resilient adults (Ahmad et al. 2010; Alim et al. 2008; Bonanno 2004) have identified a range of psychosocial factors associated with successful adaptation to stressful or traumatic events. For example, the ability to simultaneously experience both positive and negative emotions when confronted with a high-stress situation increases flexibility of thinking and problem solving and can buffer individuals from developing stress-induced adverse consequences (Fredrickson 2001; Ong et al. 2006). Likewise, optimism has been associated with resilience to stress-related disorders, including alcohol use disorders (Ahmad et al. 2010; Alim et al. 2008).

The ability to focus attention on performing and completing tasks was identified as a protective factor against substance use.

Unlike personality characteristics associated with increased risk for substance use disorders (e.g., impulsivity, novelty seeking, and negative emotionality), positive emotionality, the tendency to experience positive mood frequently, was found to be associated with resilience to substance use in a large longitudinal study of public school students followed from late childhood through midadolescence (Wills et al. 2001). In this study, positive emotionality was found to buffer the effects of parent–child conflict and of parental and peer substance use on adolescent substance use. The ability to focus attention on performing and completing tasks was identified as a protective factor against substance use (Wills et al. 2001). The ability to focus attention might relate to the capacity to cope by planning and problem solving in times of stress, both types of coping styles characteristic of resilient individuals (Southwick et al. 2005).

Veenstra and colleagues (2007) examined the impact of coping style on alcohol use in response to stressful life events in a sample of 1,608 men and 1,645 women drawn randomly from the Dutch Lifestyle and Health Study (Veenstra et al 2007). Individuals who scored high on emotion coping, a coping style focused on feelings and emotional content to cope with stress, used more alcohol when experiencing a negative life event, compared with those who scored low on emotion coping. Alcohol use in times of stress did not vary by cognitive or by action coping, but the study found that cognitive coping and having more social contacts was linked to lower alcohol use in general. Another study of more than 1,300 adult drinkers in the general population from a New York county found stress-induced drinking in a subset of men (but not women) who scored high on avoidance coping and on positive expectancy from alcohol (Cooper et al. 1992). Men with low-avoidance coping and low expectancy from alcohol, on the other hand, actually showed a negative relationship between stressful life events and alcohol use. Of note, low avoidance coping has been linked to stress resilience in general, in several other studies (Alim et al. 2008; Carver et al. 1997).

Neurochemistry of Resilience

"Allostasis" refers to the dynamic process through which the body adapts to daily stressors and maintains homeostasis (Sterling and Eyer 1988). Sudden

stressful events trigger the release of the "flight-or-fight" hormones (i.e., catecholamines) and other stress hormones in the brain, preparing the organism to cope with stress and avert harm. This process is mediated by a stress circuit (see figure 1), which is consistently implicated in stress-related disorders such as mood and anxiety disorders and addictive disorders. Interindividual variability in stress resilience results from differences in the coordinated stress response. This response comprises the function and interactions of numerous hormones, neurotransmitters, and neuropeptides, some of which are discussed below.

HPA Axis

The HPA axis is a system regulated by a complex negative-feedback system. CRF, released by the hypothalamus in response to stress, triggers the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. This process leads to the synthesis and release of cortisol by the adrenal cortex. Cortisol secretion acutely facilitates cognitive, metabolic, immunologic, and behavioral adaptations to stress. It also results, however, in "allostatic overload" when stress becomes chronic or overwhelming (McEwen 2003). Resilience is maintained when the stress response is both activated and terminated efficiently. The adaptive responses of the HPA axis are thought to involve an optimal balance of the cortisol-binding receptors GR and mineralocorticoid receptor (de Kloet et al. 2005, 2007).

Studies showing lower plasma levels of ACTH but not cortisol in men with a family history of alcoholism (Dai et al. 2007; Gianoulakis et al. 2005) suggest that HPA axis dysfunction might predate the onset of alcoholism. Long-term alcohol abuse is associated with increased extrahypothalamic CRF signaling and dampened HPA axis responsivity (Richardson et al. 2008). Increases in extrahypothalamic CRF contribute to negative emotional states during abstinence, increasing risk for relapse (Koob and Le Moal 2008). In a recent study, researchers asked alcoholics who had been abstinent for 1 month to imagine a relaxing situation of their choice while listening to a previously recorded audiotape of this situation. A greater cortisol-to-corticotropin ratio (i.e., higher adrenal sensitivity) during this relaxed state was found to predict a shorter time to alcohol relapse, thus suggesting that new treatments aimed a decreasing adrenal sensitivity could reduce relapse rates (Sinha et al. 2011).

Norepinephrine

During the acute stress response, the hormone norepinephrine (NE) is released through direct projections from the brain site where NE is synthesized (i.e., locus coeruleus) and other brain stem nuclei (i.e., structures that act as transit points for brain signals) into the amygdala, hippocampus, nucleus accumbens, prefrontal cortex (PFC), and other brain areas mediating emotional responses. Several studies have linked abnormal regulation of brain NE systems to stress disorders (Krystal and Neumeister 2009; O'Donnell et al. 2004). As drug dependence develops, levels of the neurotransmitter dopamine decrease and the NE stress system in the brain is activated, contributing to "stress-like states" and increased vulnerability to stressors during periods of abstinence (Koob and Le Moal 2008). In combi-

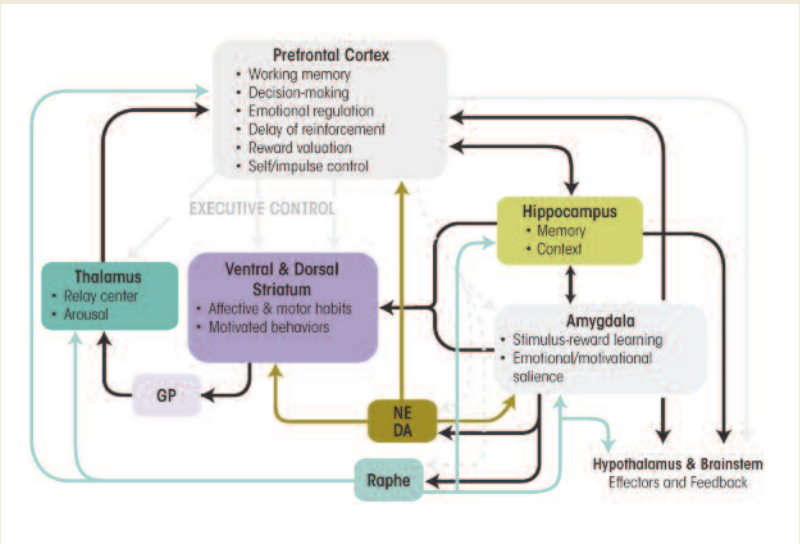

Figure 1 Norepinephrine (NE) and dopamine (DA) are the principle chemical messengers employed in central and peripheral sympathetic synapses, and the human NE transporter rapidly clears NE and DA from the synaptic cleft via efficient transport system-attenuating signaling, recycling 90 percent of these synaptic monoamines. NE neurons innervate nearly all parts of the neuroaxis, with the locus coeruleus (LC) being responsible for most of the NE in the brain. NE exerts neuromodulatory effects on the cellular activity of post-synaptic target neurons in many brain circuits, thereby moderating synaptic transmission in target circuits including the thalamus, prefrontal-cortex (PFC), ventral striatum (via PFC), and amygdala, which have been implicated in substance use disorders. The widespread and divergent anatomical organization positions the NE system to be involved in widely varying functions including responses to stress, which alters both the electrophysiological activity of NE neurons in the LC and the release of NE in the terminal regions of these cells, as well as crucial cognitive functions, including attention and arousal. NE mediates many of the adaptive and maladaptive consequences of stress exposure, implicating this system in a variety of abnormal behaviors including alcohol dependence.

nation with CRF, NE also might contribute to the consolidation of emotional memories associated with drug use in the amygdala (Koob et al. 2009).

Stress resilience may be enhanced through the regulation of NE system responsiveness, which is mediated through effects on the NE transporter on catecholamine receptors (i.e., α2 adrenoreceptors), as well as interactions between the NE and other neurobiologic systems, such as the dopamine and serotonin systems (Krystal and Neumeister 2009). For example, animal studies have shown that PFC NE nerve cell projections (i.e., axons) have a latent capacity to enhance synthesis and recovery of transmitter, which might underlie the capacity to adapt to stress (Miner et al. 2006). This mechanism deserves further study in humans with positron emission tomography (PET), which uses positron-emitting radiotracers to show where and how compounds act in the brain (Ding et al. 2005). Other targets include the α2a and α2c receptors, which have complementary roles in the regulation of stress responses (Small et al. 2000). Yohimbine, a drug that blocks the α2 receptors (i.e., a receptor antagonist), increases alcohol self-administration and induces reinstatement of alcohol seeking (Le et al. 2005; Marinelli et al. 2007). The recent finding that an α2c receptor polymorphism (Del322-325) reduces feedback inhibition of sympathetic NE release (Neumeister et al. 2005) as well as evidence from studies in mice bred to have an inactivated α2c receptor (i.e., knockout mice) (Sallinen et al. 1999), suggest that interventions targeting this receptor might modulate stress and anxiety responses.

Serotonin

The serotonin (5-HT) system, which consists primarily of neurons from the dorsal raphe nuclei that project widely throughout the brain (including the amygdala, ventral striatum, and PFC), is involved in the regulation of stress and anxiety. Serotonin has an important role in promoting neuroplasticity in the central nervous system, both during development and in adulthood. Serotonin also regulates the neurochemical effects of drugs of abuse, including alcohol, and is involved in modulating impulsivity, known to increase risk for alcohol and drug abuse (Kirby et al. 2011). The 5-HT system is itself modulated by drugs of abuse. For example, alcohol administration elevates 5-HT levels in the nucleus accumbens, ventral tegmental area (VTA), amygdala, and hippocampus, an effect that is more pronounced in alcohol-preferring rats. Reduced activity of the 5-HT system might contribute to depression during withdrawal and increase vulnerability to relapse (Kirby et al. 2011). In studies of macaques, differential function of the 5-HT system in interaction with early life stress was found to affect alcohol consumption: peer-reared female macaques with a specific variant (i.e., the l/s genotype) of the serotonin transporter polymorphism showed higher levels of ethanol preference and increased consumption over time (Barr et al. 2004).

The 5-HT system is extremely complex, including at least 14 receptor subtypes. Of these receptors, the 5-$HT1_A$, 5-$HT1_B$, 5-$HT2_A$, and 5-$HT2_C$ receptors are well understood through research on anxiety regulation in both animals and humans (Krystal and Neumeister 2009). The 5-$HT1_A$ receptor is thought to counteract the deleterious effects of 5-$HT2_A$ receptor activation (i.e., the disruption of brain cell creation), mediated by increased release of the neurotransmitter glutamate and direct glucocorticoid effects (Hoebel et al 2007). Restrained function of another 5-HT receptor, $5HT1_B$, might be central to resilient stress responses by enhancing synaptic availability of 5-HT in the amygdala and other cortical regions as well as promoting dopamine release in the ventral striatum (Clark and Neumaier 2001; Krystal and Neumeister 2009; Sari 2004) (see figure 2).

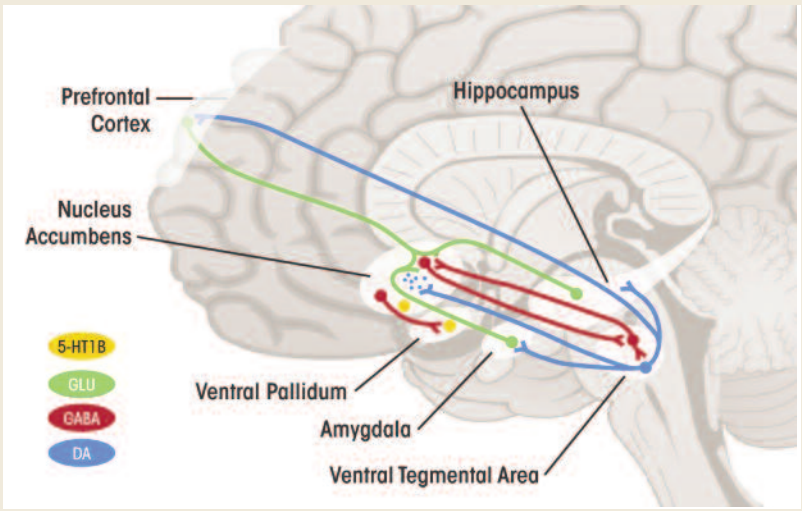

Figure 2 Alterations in serotonin 1B receptor ($5HT_{1B}R$) function might contribute to alcohol dependence by influencing not only serotonin (5HT) input to the ventral striatum via the receptors' role as 5HT terminal autoreceptors,[1] but also dopaminergic input to the striatum via the role of these receptors as heteroreceptors[2] on GABA terminals within the ventral tegmental area, and glutamatergic activity within the ventral striatum via heteroreceptors on corticofugal projections.

[1] Autoreceptor: A site on a neuron that binds the neurotransmitter released by that neuron, which then regulates the neuron's activity.
[2] Heteroreceptor: A site on a neuron that binds a modulatory neuroregulator other than that released by the neuron.

The role of this receptor subtype in addiction disorders recently was studied in humans. The report demonstrated that alcohol dependence in humans, like in rodent models, is associated with increased levels of ventral striatal 5-HT_{1B} receptors (Hu et al. 2010). Additional research is necessary to understand the complex function of the 5-HT system. However, these findings suggest possible novel targets for the treatment of stress-related disorders and, most important, addiction disorders.

Dopamine

Dopaminergic neurons in the ventral tegmental area (VTA) of the midbrain project to the nucleus accumbens and other limbic areas to form the mesolimbic dopamine system, the most studied reward circuit. Dopamine neurons are activated in response to reward or the expectation of reward, and generally are inhibited by aversive stimuli. Dopamine signaling is central to the onset of addiction, as well as to the transition to dependence in interaction with other neurotransmitter systems (Ross and Peselow 2009). Drugs of addiction trigger large but brief increases in extracellular dopamine in the nucleus accumbens. Over time, chronic drug use downregulates dopamine receptors and dopamine release, leading to decreased sensitivity to natural rewards, such as food and sex, and leading also to further drug use (Volkow et al 2010).

Although findings from animal studies suggest that early-life stress can lead to long-lasting changes in gene expression in the mesolimbic dopamine pathway, ultimately increasing vulnerability to addictive disorders, not all individuals with a history of childhood abuse develop addictive or other disorders, thereby stressing the role of protective factors such as genetic variants conferring resilience (Enoch 2010).

Findings from several studies suggest that higher dopamine D2 receptor availability in the striatum might promote resilience to alcohol use disorders. In a study of unaffected members of alcoholic families, higher striatal dopamine D2 receptor availability was associated with higher positive emotionality, discussed above as a protective factor against alcohol use disorders (Volkow et al 2006). Other studies found that higher striatal dopamine D2 receptor availability was associated with resistance to the reinforcing effects of stimulants in healthy volunteers (Volkow et al. 1999, 2002) and in rats (Thanos et al. 2008).

NPY

NPY, a 36–amino acid peptide, is widely distributed in the brain. NPY has anxiety-reducing properties in rodents and is thought to enhance resilience to stress in humans (Feder et al. 2009; Morgan et al. 2000). Evidence from animal and human studies suggests that NPY has a key role in regulating alcohol intake, dependence, and withdrawal. Mice genetically modified to overexpress NPY consume less alcohol (Thiele et al. 1998), and administration of NPY into the cerebral ventricles of the brain (i.e., intracerebroventricular infusion) reduces alcohol consumption in alcohol-preferring rats (Thorsell 2007). Infusion of NPY into the central nucleus of the amygdala has been shown to normalize both anxiety behaviors and alcohol intake, suggesting that NPY might work by modulating anxiety responses (Zhang et al. 2010). In rhesus macaques exposed to early life stress, and in human studies, certain NPY gene polymorphisms are associated with differential susceptibility to alcohol or cocaine dependence (Koehnke et al. 2002; Lindell et al. 2010; Mottagui-Tabar et al. 2005; Wetherill et al. 2008).

Endocannabinoids

An emerging body of evidence suggests an important role for the endogenous cannabinoid (eCB) system and specifically the CB_1 receptor in alcohol-related behaviors (for review, see Basavarajappa 2007). To date, however, only peripheral measures of eCB function have been collected in living humans with alcohol dependence (AD) (Mangieri et al. 2009), and no human in vivo data on the potentially critical role of the brain CB_1 receptor in AD have been collected yet. At a neurobiological level, studies show impairments in decision making in alcohol-dependent patients (Dom et al. 2006), which is associated with altered functions in a cortico-limbic-striatal circuit, including the amygdala, hippocampus, anterior cingulate cortex, insula, and the ventral striatum. Three sets of factors are thought to be responsible for high alcohol relapse rates. First, individual differences in the positive, reinforcing properties of alcohol are known to increase risk of alcoholism and possibly alcohol relapse (Schuckit and Smith 1996). Second, stimuli previously associated with alcohol use and its physiological and subjective effects become paired with alcohol and are thought to serve as "conditioned cues" that can increase alcohol craving and subsequent alcohol use (O'Brien et al. 1998). Finally, stress has been found to increase the risk of alcohol relapse (Brown et al. 1990; Miller et al. 1996; Sinha 2001). All three factors can be linked to the eCB system and its attending CB_1 receptor and increasing evidence derived from animal studies suggests a role of the eCB system in alcohol-related behaviors (Vinod and Hungund 2006).

Such research suggests that upregulation of CB_1 receptor–mediated G-protein signaling in a brain circuit that mediates AD susceptibility (involving the amygdala, hippocampus, ventromedial prefrontal cortex, insula, and ventral striatum) (Sullivan and Pfefferbaum 2005) might contribute to the increased alcohol consumption in patients with chronic AD. For example, CB_1 inactivation (Hungund et al. 2003; Naassila et al. 2004; Poncelet et al. 2003; Thanos et al. 2005) and pharmacological manipulation of CB_1 receptor function (Femenia et al. 2010; Maccioni et al.; Maccioni et al. 2008; Malinen and Hyytia 2008) result in reduced voluntary alcohol intake. In addition, administration of an agent that binds to the CB_1 receptor (i.e., a CB_1 receptor agonist) (Colombo et al. 2002; Gallate et al. 1999; Vinod et al. 2008*b*) enhances alcohol consumption.

In contrast, acute, short-term alcohol intoxication is associated with elevated eCB levels (Basavarajappa et al. 2006; Blednov et al. 2007; Vinod et al. 2008*a*), reduced activity of the enzyme fatty acid amide hydrolase (FAAH), and reduced CB_1 receptor–mediated G-protein signaling (Vinod et al. 2011). This mediates the activation of the mesolimbic dopaminergic system (Cheer et al. 2007; Hungund et al. 2003), which has been extensively studied in alcohol dependence. Evidence suggests a functional interaction between these systems, which might be associated with the reinforcing effects of alcohol and therefore may be an important mechanism in the etiology of alcohol dependence. Findings in animal studies recently have stimulated interest in the therapeutic potential of enhancing eCB signaling, with research in humans having just begun (Hill et al. 2009). However, an accumulating body of evidence suggests that the eCB system, and in particular its attending CB_1 receptor, provides novel leads for treatment development in alcohol dependence (Bailey and Neumeister 2011).

Behavioral Interventions to Enhance Resilience

To date, most studies on resilience have been conducted in clinical populations with people exposed to traumatic life events as a prototype of stress-related disorders. However, these studies also can inform the development and implementation of behavioral interventions to address alcohol dependence. This is a critical application because the ultimate goal of research attempting to delineate a range of psychological, neurochemical, and brain circuitry mechanisms underlying resilience is the development of strategies and interventions aimed at enhancing resilience in the face of stress, which is of particular relevance for people struggling with alcohol dependence. As related to alcohol dependence, improving resilience would influence cognitive and emotional control in the face of stress, resulting in the ability to weather cravings without using alcohol, mindfulness to be aware of impulsive behavior and potentially avoid impulsive behaviors associated with alcohol use, and the development of prosocial behavior and interpersonal relations that could serve to support the individual in the face of stress and prevent alcohol use. Several cognitive and behavioral interventions have been developed in an effort to develop these capacities. These interventions, which include various forms of cognitive and behavioral psychotherapies (Butler et al. 2006; Marlatt 2001), mindfulness-based stress reduction (e.g., Astin 1997; Shapiro et al. 1998; Teasdale et al. 2000) and other therapeutic approaches, aim to help prevent the onset or minimize the extent of alcohol use behaviors. In addition, therapeutic approaches based on positive psychology might also help promote psychological resilience (e.g., Seligman and Csikszentmihalyi 2000) and are currently being evaluated for their effectiveness in addressing alcohol dependence.

Researchers have hypothesized that the chronic nature of addiction disorders is rooted in the neurotoxic effects of stress on the brain.

Taken together, interventions aimed at enhancing resilience to stress that focus on developing cognitive reappraisal skills, fostering mindfulness, and facilitating social interaction that results in enhanced social support could be particularly effective in helping people cope with stress and preventing the onset of alcohol use problems or relapse. Indeed, cognitive–behavioral models of addiction and relapse treatment such as those provided by Marlatt and colleagues (e.g., Marlatt 2001) highlight the role of experiencing negative affect as a primary trigger for using alcohol and relapsing. Mindfulness skills can be particularly useful in helping an individual cope with negative affect in the moment without resorting to the use of substances. Moreover, the attributions that individuals make upon relapsing (whether the attribution for use is internal and stable: "I just can't handle stress and I'm bound to keep using"—versus external and unstable: "This was really stressful and difficult to deal with, and I decided to take the easy route this time") can influence whether the relapse develops into a full-blown relapse or remains an isolated event. Cognitive reappraisal of these situations and the attributions that individuals make of their alcohol use can thus be of great importance in developing resilience in the treatment of alcohol use disorders.

Conclusions and Future Directions

Despite extensive research and knowledge regarding their serious adverse consequences, addiction disorders continue to contribute to the top preventable causes of death and morbidity in the United States (Centers for Disease Control and Prevention 2003). The mechanisms underlying the persistent and compulsive engagement in these behaviors remain poorly understood. Based on previous evidence, researchers have hypothesized that the chronic nature of addiction disorders is rooted in the neurotoxic effects of stress on the brain. These effects undermine the neuroplasticity within networks required for the recovery process to take place. As a result, mechanisms of resilience are crucial to the understanding of neuroadaptive potential and its behavioral consequences. This is an important topic of current research, which stands at a unique crossroad in the study of addiction disorders. The explosion in the field of molecular and cellular neuroscience calls for interdisciplinary,

collaborative team-based approaches. A greater understanding of the neurobiology of stress and resilience, as well as its implications on the neurobiology of addictions, is crucial to the prevention of such disorders and to the development of evidence-based treatment strategies. ■

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Stress and the HPA Axis

Role of Glucocorticoids in Alcohol Dependence

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Stress has long been suggested to be an important correlate of uncontrolled drinking and relapse. An important hormonal response system to stress—the hypothalamic–pituitary–adrenal (HPA) axis—may be involved in this process, particularly stress hormones known as glucocorticoids and primarily cortisol. The actions of this hormone system normally are tightly regulated to ensure that the body can respond quickly to stressful events and return to a normal state just as rapidly. The main determinants of HPA axis activity are genetic background, early-life environment, and current life stress. Alterations in HPA axis regulation are associated with problematic alcohol use and dependence; however, the nature of this dysregulation appears to vary with respect to stage of alcohol dependence. Much of this research has focused specifically on the role of cortisol in the risk for, development of, and relapse to chronic alcohol use. These studies found that cortisol can interact with the brain's reward system, which may contribute to alcohol's reinforcing effects. Cortisol also can influence a person's cognitive processes, promoting habit-based learning, which may contribute to habit formation and risk of relapse. Finally, cortisol levels during abstinence may be useful clinical indicators of relapse vulnerability in alcohol-dependent people. **KEY WORDS: Alcohol dependence; problematic alcohol use; alcohol use disorders; alcohol abstinence; relapse; stress; stress response; stress hormones; hypothalamic–pituitary–adrenal axis; glucocorticoids; cortisol; brain reward pathway**

Stress, generally defined as any stimulus that disrupts the body's internal balance (i.e., physiological homeostasis), has long been suggested to be an important correlate of uncontrolled alcohol consumption or relapse to drinking following a period of abstinence. Large epidemiological studies have reported that a variety of stressors are associated with increased alcohol consumption and binge drinking. These include hazardous and demanding work environments, legal stress, family stress (e.g., unhappy marriage and divorce), and low income (Richman et al. 1996; Rospenda et al. 2000; San Jose et al. 2000; Vasse et al. 1998). Likewise, the Health and Retirement Study found an association between stress from retirement and divorce and increased alcohol intake (Perreira and Sloan 2001). Studies also have shown that people experiencing more severe or highly threatening social stress following alcoholism treatment have higher rates of relapse compared with people not experiencing such stress (Brown et al. 1990; Noone et al. 1999). On the other hand, prospective and human laboratory studies exploring the relationship between stress, alcohol craving, and relapse have found mixed results, with more recent research suggesting that several factors moderate the effects of stress on alcohol consumption (e.g., Breese et al. 2011; Brennan et al. 1999; Fox et al. 2008; Helzer et al. 2006; Sinha 2007; Sinha and Li 2007; Thomas et al. 2011).

It remains uncertain how stress, per se, might influence vulnerability to alcohol use disorders (AUDs). However, production of the stress hormone cortisol, which is triggered by stress-induced activation of a hormonal system known as the hypothalamic–pituitary–adrenal (HPA) axis, is thought to be involved. The HPA axis is one of the main stress response pathways and has been studied extensively in relation to alcohol use (Wand 2008). Over 20 years of research has demonstrated that altered HPA axis regulation is associated with problematic alcohol use and dependence and that the nature of this dysregulation varies with respect to the stages of progression toward alcohol dependence. The finding that HPA axis dysregulation and alcohol misuse tend to co-vary has implied a "guilt-by-association" relationship—that is, that abnormal variations in stress-related cortisol production are a risk factor for developing alcoholism in the first place (Wand et al. 1993). A recent review of studies on youth and adolescents similarly suggests that HPA axis dysfunction and exposure to stress are critical components that interact to convey risk for developing AUDs (Schepis et al. 2011).

As with mood and affective disorders, many researchers consider alterations in HPA axis function crucial for understanding the underlying brain mechanisms of substance use disorders. In contrast to mood and affective disorders, however, alcohol dependence has a biphasic effect on HPA axis dynamics as a person traverses through the various phases of heavy hazardous drinking, including dependent drinking, withdrawal, abstinence, and relapse. Generally speaking, these developmental stages seem to be mirrored by a shift between hyper- and hyporesponsiveness of the HPA axis to stressful events (Rose et al. 2010). For example, hyperresponsiveness has been identified in people with a family history of alcoholism (Uhart et al. 2006; Zimmermann et al. 2004*a*,*b*), a population that is at increased risk for alcohol dependence (Windle 1997). This observation raises the question whether heightened stress responsivity is clinically meaningful to the development of alcoholism. This view is supported by studies showing that cortisol responsivity correlates with the activity of a brain system, the mesolimbic dopaminergic pathway, which is a central neural reward pathway (Oswald et al 2005; Wand et al. 2007). With transition to alcohol dependence, compensatory allostatic mechanisms result in injury to HPA axis function and elevation of stress peptide levels (e.g., corticotropin-releasing factor [CRF]) in brain regions outside the hypothalamus. The term allostasis refers to the process through which various biological processes attempt to restore homeostasis when an organism is threatened by various types of stress in the internal or external environment. Allostatic responses can involve alterations in HPA axis function, the nervous system, various signaling molecules in the body, or other systems. Allostatic alterations in HPA axis function have been posited to, among other things, injure brain reward pathways, contribute to depressed mood (i.e., dysphoria) and craving, and further contribute to the maintenance of problem drinking behavior.

This article provides an overview of the clinical evidence for HPA axis and glucocorticoid dysfunction across the developmental phases of alcoholism and explores whether this dysfunction is causally related to, or a consequence of, alcohol dependence. The article describes behavioral and physiological pathogenesis resulting from dysregulation of basal and reactive HPA axis activity. This discussion primarily focuses on human studies and studies that specifically address the glucocorticoid activation component of the stress response. The article also discusses whether these findings have potential predictive value and whether altered glucocorticoid function, regardless of etiology, may serve as a useful clinical marker for the progression of alcohol dependence and treatment prognosis. The review will not address the important role that extrahypothalamic CRF pathways play in mediating the relationship of stress and reward dysfunction (for a review of this issue, see Koob 2010).

Physiology of the HPA Axis

The body responds to stress with self-regulating, allostatic processes aimed at returning critical systems to a set point within a narrow range of operation that ensures survival. These self-regulating processes include multiple behavioral and physiological components. Perhaps the best-studied component of the stress response in humans and mammals is activation of the HPA axis (see figure 1). Neurons in the paraventricular nucleus (PVN) of the hypothalamus release two neurohormones—CRF and arginine vasopressin (AVP)—into the blood vessels connecting the hypothalamus and the pituitary gland (i.e., hypophysial portal blood). Both hormones stimulate the anterior pituitary gland to produce and secrete adrenocorticotropic hormone (ACTH) into the general circulation. The ACTH, in turn, induces glucocorticoid synthesis and release from the adrenal glands, which are located atop the kidneys. The main glucocorticoid in humans is cortisol; the main glucocorticoid in rodents, which frequently are used as model systems to investigate the relationship between stress and alcohol use, is corticosterone. Hypothalamic activation of the HPA axis is modulated by a variety of brain signaling (i.e., neurotransmitter) systems. Some of these systems have inhibitory effects (e.g., γ-aminobutyric acid [GABA] and opioids), whereas others have excitatory effects (e.g., norepinephrine and serotonin) on the PVN. Thus, the central nervous system (CNS) and the hormone (i.e., endocrine) system are tightly interconnected to coordinate glucocorticoid activity.

To protect against prolonged activity, the HPA system is carefully modulated through negative-feedback loops designed to maintain predetermined hormone levels (i.e., set points) and homeostasis. To this end, secretion of CRF, AVP, and ACTH in part are controlled by sensitive negative feedback exerted by cortisol at the level of the anterior pituitary gland, PVN, and hippocampus. There are two types of receptors for cortisol—mineralocorticoid (type-I) and glucocorticoid (type-II) receptors—both of which participate in the negative-feedback mechanisms. Cortisol binds more strongly (i.e., has higher binding affinity) for the mineralocorticoid receptors (MRs)[1] than the glucocorticoid receptors (GRs). Because of this difference in binding affinity, the MRs help maintain the relatively low cortisol levels circulating in the blood during the normal daily (i.e., circadian) rhythm. Only when the cortisol concentration is high (e.g., during a stressful situation) does it bind to the GRs with lower affinity; the resulting activation of the GRs terminates the stress response. This delicate negative feedback control mechanism maintains the secretion of ACTH and cortisol within a relatively narrow bandwidth. This is an extremely important homeostatic mechanism because too much or too little exposure

[1]Cortisol has similar affinity to the MR as does the mineralocorticoid aldosterone, which helps regulate kidney function.

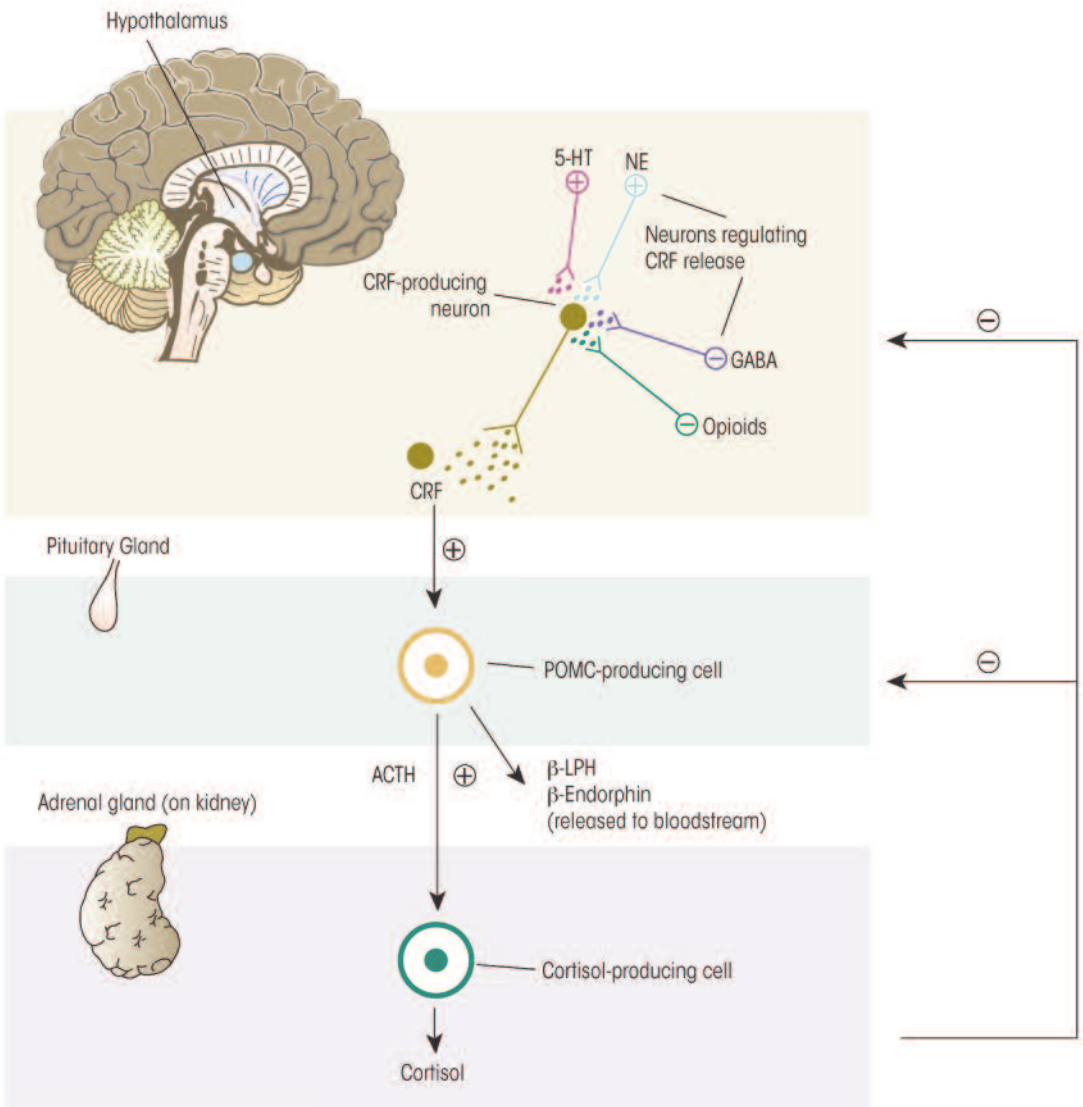

Figure 1 The major components of the stress response mediated by the hypothalamic–pituitary–adrenal (HPA) axis. Both alcohol and stress can induce nerve cells in one brain region (i.e., the hypothalamus) to produce and release corticotropin-releasing factor (CRF). Within the hypothalamus, CRF stimulates the release of a hormone that produces morphine-like effects (i.e., β-endorphin). CRF also is transported to a key endocrine gland, the anterior pituitary gland. There, CRF stimulates production of a protein proopiomelanocortin (POMC). POMC serves as the basis for a number of stress-related hormones, including adrenocorticotropic hormone (ACTH), β-lipotropin (β-LPH), and β-endorphin. ACTH stimulates cells of the adrenal glands to produce and release the stress hormone cortisol. When cortisol levels reach a certain level, CRF and ACTH release diminishes. Other neurons releasing serotonin (5-HT), norepinephrine (NE), γ-aminobutyric acid (GABA), or endogenous opioids also regulate CRH release.

NOTE: ⊕ = excites; ⊖ = inhibits.

to cortisol can have adverse consequences to health and well being.

Growing evidence suggests that a protein, FK506 binding protein 5 (FKBP5), regulates GR sensitivity. Binding of this protein to the GR reduces the receptor's affinity for cortisol and its movement (i.e., translocation) to the nucleus. A genetic variation in FKBP5 is associated with enhanced expression of the protein following GR activation. This leads to more GR resistance, diminished negative feedback, and prolonged stress hormone activation following a stressor (Binder et al. 2004; Wochnik et al. 2005).

Physiological Actions of Glucocorticoids

Glucocorticoids are a class of steroid hormones that are essential for the organism to survive. Cortisol, the main glucocorticoid in humans, has been placed in this class because of its effects on the metabolism of the sugar glucose, where its primary function is to increase blood glucose levels by inducing production of additional glucose molecules (i.e., gluconeogenesis). Cortisol also modifies fat and protein metabolism to support the nutrient requirements of the CNS during stress. However, cortisol also has many other wide-ranging effects when it binds to GRs. For example, it influences cardiovascular function, immunologic status (i.e., inflammatory reactions), arousal, and learning and memory; all of these systems therefore are affected when the HPA axis is activated in response to stress.[2] Thus, cortisol helps maintain or can increase blood pressure by increasing the sensitivity of the blood vessels to signaling molecules, catecholamines. In the absence of cortisol, widening of the blood vessels (i.e., vasodilation) and hypotension occurs. The anti-inflammatory effects of cortisol are brought about by reducing proinflammatory cytokine and histamine secretion and stabilizing the membranes of cell components, lysosomes.

One of the most important actions of cortisol in the context of alcohol use and the stress response is its role in modifying learning and memory. Both stress and exposure to cortisol can transiently block memory retrieval (van Stegeren 2009), with retrieval of emotional memory more strongly affected than that of neutral memory. Of interest, both cortisol and stress also enhance memory consolidation; this process generally favors consolidation of emotionally arousing information, facilitating habit-based learning. Consistent with the multiple-systems theory to memory organization in the mammalian CNS, studies have identified unique roles for various brain regions in learning and memory. For example, "cognitive" learning and memory is associated with activation of brain circuits in the hippocampus, whereas "habit" learning and memory is associated with activation of the dorsal striatum and the basolateral amygdala (BLA). In addition, nerve fibers projecting from the BLA modulate memory processes occurring in other brain structures. The implications of the fact that cortisol selectively affects emotionally charged memory and habit learning are discussed below.

Determinants of HPA Axis Activity and Cortisol Exposure

Correct regulation of cortisol levels is necessary for survival, and too little or too much cortisol exposure can result in serious harm. Therefore, both basal and stress-induced cortisol levels are maintained carefully. A healthy stress response is characterized by a quick rise in cortisol levels, followed by a rapid decline with the termination of the stressful event. When the organism is burdened by cumulative stress, however, the cortisol burden increases. This results in wear and tear on the organism from excessive exposure to the catabolic properties of glucocorticoids, stress peptides, and proinflammatory cytokines. This burden taxes the organism and can influence the development of neuropsychiatric and metabolic disorders. It therefore is essential to understand the systems that regulate cortisol production.

Three main determinants of HPA axis activity control the amount of cortisol a person is exposed to during adulthood: genetic background, early-life environment, and current life stress. In addition, studies found that post-traumatic stress disorder (PTSD) can contribute to HPA axis disturbances.

Genetic Factors. Differences among individuals in cortisol responses to stress result from a complex interplay between genetic and environmental factors. The genetic contribution to the variability in HPA axis reactivity is believed to arise from DNA variations (i.e., polymorphisms) in the genes encoding neurotransmitters involved in HPA axis regulation. Overall, heritable influences account for approximately 62 percent of the etiological variance in basal glucocorticoid levels (Bartels et al. 2003). Recent candidate gene association studies using laboratory-based stress procedures also have implicated multiple gene variants in explaining some of the variance in cortisol responses to stress, including polymorphisms in the following genes:

- *Nr3c1*, which encodes a glucocorticoid receptor protein (Wust et al. 2004);
- *Nr3c2*, which encodes a mineralocorticoid receptor protein (DeRijk et al. 2006);
- *FKBP5* (Ising et al. 2008);
- *CRFR1*, which encodes the CRF receptor 1 protein (Clarke and Schumann 2009);
- *CRF-BP*, which encodes CRF binding protein (Wang et al. 2007);

[2] Certain tissues, however, need to be protected from cortisol, such as the kidneys, colon, and placenta. In these tissues, an enzyme ,11-β hydroxysteroid dehydrogenase type II, mediates the conversion of glucocorticoids to 11-dehydro metabolites, which are inactive.

- *GABRA6*, which encodes the GABA receptor subunit alpha-6 protein (Uhart et al. 2004);
- *OPRM1*, which encodes the mu opioid receptor protein (Chong et al. 2006); and
- *SLC6A4*, which encodes a serotonin transporter protein (Way and Taylor 2010).

It is certain that additional genes and polymorphisms will be identified in the future.

Early-Life Environment. Pre- and postnatal processes contribute to the lifelong responsiveness of the HPA axis to stressors. In animal models, prenatal ethanol exposure is associated with impaired HPA axis responsivity in adulthood (Hellemans et al. 2010; Weinberg et al. 2008), and emerging evidence suggests that these effects also occur in human infants and toddlers (Haley et al. 2006; Ouellet-Morin et al. 2010). Maternal stress during gestation also modifies HPA axis responsivity of infant and adult offspring (see Charil et al. 2010; Harris and Seckl 2010 for reviews). More recently, studies have focused on the consequences of early-childhood events on the stress response. Childhood trauma is a significant problem in the United States and is associated with mental and physical health problems in adulthood as well as with alterations in HPA axis function (Heim et al. 2009, 2010; Dong et al. 2004; Mangold et al. 2010). For example, it has been hypothesized that exposure to sexual and physical abuse in childhood during critical periods of brain development (i.e., during periods of neural plasticity) may permanently alter stress responsivity (Gillespie et al. 2009; Heim and Nemeroff 2001; Heim et al. 2001). Animal models that have studied this phenomenon have shown that certain forms of neonatal stress results in a modification (i.e., epigenetic methylation) of the glucocorticoid gene that has long-lasting effects on glucocorticoid responsivity (Weaver 2009). This alteration in stress responsivity may explain the observation that childhood adversity is a risk factor for the development of alcohol and other drug abuse (Epstein et al. 1998) as well as anxiety and depressive disorders in adulthood (Kessler et al. 1997; Safren et al. 2002).

Glucocorticoids also can alter the methylation patterns of other genes. For example, glucocorticoid administration to adolescent mice reduces methylation of the *FKBP5* gene in the hippocampus, hypothalamus, and blood, which is associated with enhanced expression of *FKBP5* and increased anxiety-like behavior (Lee et al. 2010). The investigators proposed that in addition to altering behaviors, methylation of the gene may be a marker of cortisol burden. Polymorphisms in *FKBP5* also have been associated with psychiatric disorders, such as depression and PTSD, that are characterized by alterations in HPA dynamics (Binder et al. 2004; Yehuda et al. 2009).

An emerging literature also addresses the role of early-childhood adversity on the development of AUDs (for a review, see Enoch 2010). For example, Schmid and colleagues (2010) found an interaction between stressful early-life events and a variant in the *CRFR1* gene that influenced age of drinking initiation and drinking progression in a population of 19-year-olds. Other studies demonstrated that certain variants of the *CRFR1* gene influenced cortisol responses to CRF and the synthetic glucocorticoid dexamethasone (Binder et al. 2010; Tyrka et al. 2009) and were associated with binge drinking in adolescents and total lifetime alcohol consumption in adults (Clarke and Schumann 2009; Hansson et al. 2006; Pastor et al. 2008; Treutlein et al. 2006). Thus, it seems that an interaction between the *CRFR1* gene and early-life events can modify HPA axis dynamics and risk for AUDs. It is certain that other stress gene variants also will be found to interact with environmental factors to increase the risk of AUDs.

Current Stress. Independent of prenatal and childhood stressors, periods of severe, chronic stress in adulthood, such as family- and work-related problems, combat exposure, neighborhood violence, chronic illness, or the development of neuropsychiatric disorders, alter HPA axis dynamics and increase the cortisol burden. Chronic stress triggers an allostatic shift in the normal circadian rhythm of cortisol release as well as in stress-induced cortisol levels. Thus, after chronic stress baseline cortisol levels are elevated, the body's cortisol response to acute stress is blunted, and it takes longer for stress-induced cortisol levels to return to pre-stress levels (e.g., Juster et al. 2010; McEwen 2000; Wingenfeld et al. 2009). This allostatic injury makes the HPA axis more sensitive, resulting in higher cortisol exposure or greater cortisol burden following each stressful episode (McEwen and Gianaros 2010).

PTSD Symptomatology. A fourth potential determinant of HPA axis activity is the presence of PTSD symptoms. The HPA axis has been the main focus of neuroendocrine research in PTSD. In a meta-analysis of 37 studies involving people with PTSD, Meewisse and colleagues (2007) examined cortisol levels in people with PTSD and control subjects. These analyses found no differences in basal cortisol levels between the two groups; however, differences did exist under certain conditions or among certain subgroups of subjects. For example, people with PTSD had lower afternoon levels of cortisol than did control subjects, and women with PTSD had significantly lower cortisol levels than women without PTSD. The specific type of trauma experienced by a person also mattered. Thus, only people who had experienced physical or sexual abuse had significantly lower cortisol levels than control subjects. These findings highlight the complexity of the relationship between HPA axis activity and PTSD pathophysiology.

People with AUDs have a high prevalence of PTSD (Kessler et al. 1997); conversely, women with PTSD were 3.5 times more likely to develop alcoholism than women who did not report past trauma (Sartor et al. 2010). It is difficult to define whether the alterations in the HPA axis seen in people with PTSD by themselves modulate risk for alcoholism because, as discussed above, a history of childhood trauma also increases risk for developing PTSD as well as alcoholism (Binder et al. 2008; Epstein et al. 1998). Therefore, it is possible that exposure to trauma in early childhood may confer the initial insult to HPA axis regulation that later influences the interaction between PTSD and alcohol use (Yehuda et al. 2010). This view is consistent with the finding that people with a flattened cortisol response following trauma had a higher risk of developing PTSD symptoms than did those with normal cortisol levels (e.g., Aardal-Eriksson et al. 2001; Anisman et al. 2001). It remains unclear, however, whether the lower levels of circulating cortisol preceded the traumatic event (Yehuda et al. 2010).

Regardless of whether an underlying HPA axis dysregulation precedes PTSD symptomatology, evidence suggests that dysregulation occurs through increased sensitivity of the negative feedback mechanisms regulating the HPA axis, resulting in lower circulating cortisol levels. Yehuda and coworkers (2009) examined the expression of all genes active in whole-blood samples as well as cortisol levels in people with and without PSTD. This analysis identified 17 genes whose expression differed between people with and without PTSD. Several of the uniquely expressed genes are involved in HPA axis function. For example, the *FKBP5* gene, which serves as a modulator of GR sensitivity, showed reduced expression in people with PTSD, consistent with enhanced GR responsiveness. Moreover, statistical analyses found that *FKBP5* expression was predicted by cortisol levels when PTSD severity also was taken into consideration (Yehuda et al. 2009). Of interest, this profile of HPA axis dysregulation is distinct from that seen with other psychiatric disorders, such as depression (Handwerger 2009). Taken together, it seems likely that dysregulation of the HPA axis associated with PTSD interacts with epigenetic and environmental influences (Yehuda et al. 2010) and that this interaction translates into increased risk for the development of AUDs.

The HPA Axis and Alcoholism

HPA Axis Dynamics in People at Risk for AUDs

Altered HPA axis responsivity may be present before alcohol exerts its toxic effects on the CNS and may contribute to initial vulnerability to alcoholism. This vulnerability risk likely is a result of gene–environment interaction (Clarke et al. 2008; Schepis et al. 2011). The current state of knowledge stems from an early and large body of research suggesting that people who have alcoholic family members (i.e., who are family-history positive [FHP] for alcoholism) may be more likely to develop the disorder than those with no such family history (i.e., who are family-history negative [FHN] for alcoholism) (Windle 1997). This risk seems to be linked to abnormal HPA activity (e.g., Dai et al. 2002; King et al. 2002; Sorocco et al. 2006; Uhart et al. 2006; Wand et al. 1998, 1999*a,b*), although the relationships appear complex. Laboratory findings have been mixed and may depend on several factors, such as which type of stressor is used, whether basal or reactive HPA response is measured, and how cortisol is stimulated. The first studies comparing HPA axis responsivity in FHP and FHN people assessed cortisol levels in response to an agent that can block the opioid receptors (i.e., the opioid receptor antagonist, naloxone). These studies identified stronger cortisol responses to naloxone in FHP subjects than in FHN subjects (Wand et al. 1998, 1999*a,b,* 2001). These findings were replicated using another opioid receptor antagonist, naltrexone (King et al. 2002). These observations are particularly interesting because they implicate the endogenous opioid system in the interaction between HPA axis activity and alcoholism risk. This signaling system not only modulates the HPA axis but also is a pharmacological target for the treatment of alcohol dependence. Other studies using a psychosocial stressor rather than a pharmacologic stimulator such as naloxone also found a stronger HPA response in FHP than in FHN subjects (Uhart et al. 2006; Zimmermann et al. 2004*a,b*). More recent studies among infants and toddlers with prenatal alcohol exposure who also are believed to be at increased risk for alcoholism have corroborated these latter findings in male but not female children (Haley et al. 2006; Ouellet-Morin et al. 2010). Other studies, however, found blunted HPA axis function in FHP individuals (e.g., Dai et al. 2002; Sorocco et al. 2006).

HPA Axis Dynamics During Intoxication and Withdrawal

As with stress, acute alcohol consumption also directly and indirectly activates the HPA axis by resulting in elevated levels of glucocorticoids (Richardson et al. 2008). In fact, alcohol and other drugs of abuse have been described as a physiological stressor because they can activate the HPA axis. In social drinkers, acute doses of alcohol usually increase cortisol levels, particularly if blood alcohol levels exceed 100 mg percent (Waltman et al. 1993). At some point during the transition from social drinking to alcohol dependence and abstinence, however, the HPA axis becomes dysregulated. For example, King and colleagues (2006) found that cortisol reactivity to acute alcohol administration is attenuated in heavy, hazardous drinkers compared with light, social drinkers. This observation may be related to the general process of tolerance that emerges during heavy hazardous drinking. It is important to note that the subjects in this study were binge drinkers—which reflects a pattern of drinking frequently associated with adverse consequences—but

were not alcohol dependent, suggesting that alterations in the HPA axis may begin even before dependence develops.

The onset of alcohol dependence, however, is accompanied by bouts of elevated cortisol levels in the blood (i.e., hypercortisolism) as the drinker cycles though repeated episodes of alcohol intoxication and the stress of withdrawal (Adinoff et al. 1998; Wand and Dobs 1991). This transition to alcohol dependence is accompanied by an allostatic shift in HPA axis functioning, resulting in abnormally low cortisol responsivity (Koob and Le Moal 2001). Under conditions of alcohol dependence, the allostatic load—a hypothetical measure of cumulative stress—increases and burdens the organism with excessive exposure to stress hormones and peptides as well as pro-inflammatory cytokines (McEwen 2007). Increased allostatic load has been implicated not only in AUDs and other drug use disorders but also in the development psychiatric disorders (e.g., depression), metabolic syndrome, and systemic hypertension. In the context of drug use, allostatic load not only impacts the stress response via the HPA axis but also encompasses a state of reward dysregulation. At this point, the organism constantly seeks the initial rewarding effects of the drug while tolerance to those effects develops through repeated drug self-administration. This results in a dysfunctional reward system and a maladaptive response to stress. Specifically, the allostatic alterations in cortisol responsivity may have a detrimental effect on the reward systems (Wand 2008).

HPA Axis Dynamics During Abstinence

Wand and Dobs (1991) studied HPA axis function in alcohol-dependent subjects during the first week of abstinence following supervised alcohol withdrawal on a clinical research unit. Although the participants had modestly to highly significantly elevated cortisol levels in the urine during the withdrawal period, they also demonstrated blunted HPA axis responses to CRF, a medication that blocks cortisol production (i.e., metyrapone), and the ACTH analog cosyntropin immediately following alcohol detoxification. In fact, many of the alcohol-dependent subjects met diagnostic criteria for adrenal insufficiency. Other studies have corroborated these findings of elevated cortisol during the first week of withdrawal and also showed that cortisol levels decreased significantly over time, even plunging below the normal range (Esel et al. 2001; Keedwell et al. 2001; Majumdar et al. 1989).

Later in abstinence (i.e., at 2 to 6 weeks), alcoholics generally regain normal diurnal patterns of cortisol levels (e.g., Leggio et al. 2008). However, they may continue to exhibit a deficient cortisol response to psychosocial and pharmacological HPA axis stimulation for several months (Adinoff et al. 1998, 2005*a*,*b*; Anthenelli et al. 2001; Bernardy et al. 1996). Junghanns and colleagues (2007) compared HPA axis activity in early abstainers (i.e., mean abstinence 22 days) and long-term abstainers (i.e., mean abstinence 117 days). These investigators found that longer-abstaining people showed a stronger cortisol awakening response, another indicator of HPA axis function, implying that diurnal patterns of cortisol may begin to normalize over longer periods of abstinence. Whether regulation of the HPA axis returns completely to normal, and under what conditions, remains unknown.

Several factors may impact and moderate HPA axis recovery, including severity of withdrawal symptoms (Bernardy et al. 1996), severity and duration of dependence, comorbid childhood trauma (Schafer et al. 2010), and genetic factors underlying the individual stress response. The exact role of cortisol in HPA axis recovery is unclear. Coiro and colleagues (2007) examined the effect of exercise as a biobehavioral stressor in control subjects and alcoholics over an 8-week period. Consistent with other studies, ACTH and cortisol levels were significantly lower in alcoholics in the first month of withdrawal; by 8 weeks, however, the hormonal response had returned to normal. Interestingly, exercise itself can induce cortisol release (Beaven et al. 2010; Coiro et al. 2007; Usui et al. 2011) and has been investigated as an adjunct for smoking cessation with somewhat promising findings (Williams et al. 2010). This suggests that manipulation of cortisol levels may have therapeutic potential (see below). Indeed, determining the nature, extent, and time course of the attenuated HPA axis response during abstinence may have significant clinical relevance because low levels of basal cortisol and of the ACTH response may predict relapse to alcohol use during early abstinence (Adinoff et al. 1998; Junghanns et al. 2003, 2005; Kiefer et al. 2002).

No prospective longitudinal studies have examined HPA axis changes over longer periods of abstinence. One study of alcoholics who had been abstinent for a mean of 3.5 years found similar ACTH and cortisol responses compared with healthy controls in response to both psychological and pharmacological (i.e., opioid challenge) stressors (Munro et al. 2005). However, the study did not determine whether the alcoholics had recovered a normal level of HPA response with prolonged abstinence, whether they had had a normal response all along, or whether their lack of psychological comorbidity indicated that they were less affected by secondary characteristics related to a hyporesponsive HPA axis. Another study compared alcoholics who had relapsed with abstainers after one year and found that, contrary to findings during short-term abstinence, 1-year abstainers had significantly lower levels of cortisol (Walter et al. 2006). This suggests that the relationship between HPA axis activity and alcohol recovery is dynamic and changes as abstinence persists over time.

One major limitation of these studies is that most of the work has been conducted with male alcoholics; therefore, less is known regarding the HPA hyporesponsiveness during abstinence in females. Adinoff and colleagues (2010) focused on female alcoholics

and found no differences in HPA axis activity between women who had been abstinent for 4 to 8 weeks and age-matched healthy control women. Thus, HPA axis functioning over the long term and its relationship to alcohol use and recovery remains unclear and warrants further investigation.

Possible Roles of Cortisol in the Risk and Development of AUDs

Cortisol's Interaction with Dopaminergic Reward Systems

Studies in animal models have demonstrated that mesocorticolimbic dopamine pathways are involved in the brain's reward system and that the nucleus accumbens in the ventral striatum is a critical region for mediating the rewarding effects of drugs. Virtually all drugs of abuse, including alcohol, have an impact on dopaminergic activity within this brain region (Pierce and Kumaresan 2006). Imaging studies using positron emission tomography (PET) in humans have corroborated the animal findings that drugs of abuse alter mesolimbic dopaminergic activity and have helped elucidate potential neurobiological underpinnings of drug addiction (for a review, see Martinez and Narendran 2009). These and other studies in humans have shown that mesolimbic dopamine release is correlated with the positive subjective effects of the drug (Drevets et al. 2001; Hamidovic et al. 2010; Oswald et al. 2005; Volkow et al. 2002; Wand et al. 2007). However, whereas acute alcohol administration increases synaptic dopamine activity and accumulation, chronic alcohol consumption can lead to lower-than-normal dopamine levels (i.e., a hypodopaminergic state) that may motivate the drinker to seek alcohol in order to restore the normal levels of the neurotransmitter (Volkow et al. 2007). It has been postulated that elevated levels of glucocorticoids contribute to alcohol's reinforcing effects by enhancing modulation of the dopaminergic and subjective response to alcohol (e.g., Melis et al. 2009).

Glucocorticoids and stress interact with the dopamine reward system in ways that may increase vulnerability for developing addiction (Marinelli and Piazza 2002). For example, glucocorticoids play a critical role in the reinforcing effects of psychostimulants because surgical removal of the adrenal glands (i.e., adrenalectomy), which prevents cortisol production, decreases drug self-administration. Moreover, re-introduction of glucocorticoids at levels similar to those induced by stress reverses this effect (Deroche et al. 1997). In fact, acute stress and drugs of abuse, through different mechanisms, appear to converge upon a common pathway that modifies dopamine neuron output by enhancing long-term potentiation (LTP) of excitatory synapses (Saal et al. 2003) and long-term depression (LTD) of inhibitory synapses (Niehaus et al. 2010). However, these studies did not demonstrate that this effect directly was attributable to cortisol. Another study found that the magnitude of stress-induced cortisol release significantly correlates with mesolimbic dopamine release in the ventral striatum (Pruessner et al. 2004). Taken together, these studies suggest that cortisol may facilitate firing of dopaminergic neurons and, consequently, the reward circuitry and that this process is common with and specific to many drugs of abuse (Saal et al. 2003).

Glucocorticoids themselves also are believed to have reinforcing properties in rats as they seem to modulate self-administration of alcohol and increase brain sensitivity to other addictive drugs (e.g., stimulants and opioids) in the animals. A review by Piazza and Le Moal (1997) concluded that glucocorticoid administration at levels similar to those found in physiological stress responses had positive reinforcing effects. The investigators proposed that under natural conditions (e.g., during conflicts with other animals) the rewarding effects of the glucocorticoids might counteract the aversive effects of external aggressions, thereby allowing the animal to better cope with threatening situations. Such a mechanism may play a key role in fine-tuning an individual's adaptation to stress and in determining reward-related behavioral pathologies. Thus, increased levels of cortisol may have reinforcing effects, acting on the brain to perpetuate behaviors (e.g., alcohol consumption) that maintain high cortisol levels.

The interactions of the stress response and the rewarding effects of drugs also have been investigated in humans. Imaging studies using PET found that higher cortisol levels in response to amphetamine administration (Oswald et al. 2005) or to a psychosocial stressor (Wand et al. 2007) were positively associated with amphetamine-induced dopamine release in the ventral striatum. Furthermore, subjects with a high cortisol response to these stimuli reported more positive subjective drug effects after amphetamine administration than did subjects with a low cortisol response (Hamidovic et al. 2010; Oswald et al. 2005; Wand et al. 2007). These studies provide evidence that cortisol may play a role in drug reinforcement through its interactions with the dopaminergic reward pathway, which may, in turn, influence vulnerability for and maintenance of alcohol and other drug use.

Cortisol's Effect on Cognitive Processes

LTP is a process that ultimately enhances signal transmission at the synapse. This enhanced synaptic transmission, which has been observed in a variety of neural structures, is widely considered one of the leading cellular mechanisms that underlie learning and memory (Goosens and Maren 2002). As mentioned above, LTP is enhanced by stress. Cortisol has been implicated in this phenomenon because a widespread system of glucocorticoid receptors is found above the hypothalamus, for example, in the limbic system, notably the hippocampus and amygdala, and in the prefrontal cortex. This section discusses the impact of glucocorticoids

on some of the basic (e.g., learning, acquisition, and memory) and higher (e.g., decision-making) cognitive processes that may potentially underlie development of addictive behaviors. This discussion focuses on the regulatory actions of glucocorticoids on neural structures critically involved in cognitive processes related to alcoholism but does not cover the equally important reciprocal effects these structures have on regulating HPA axis function (e.g., Dedovic et al. 2009).

Optimal levels of cortisol are needed not only to meet the body's physical needs but also for learning, memory, and cognitive performance. Both too little and too much cortisol may be damaging and disruptive to memory formation, whereas normal levels of glucocorticoids protect the brain against adverse events and are essential for cognitive processes. Several studies partly may explain this paradox by describing the roles of MRs and GRs in the various stages of information processing and the context in which glucocorticoid-receptor activation takes place. The effects of glucocorticoids on brain tissue as well as cognition can turn from adaptive into maladaptive when actions via both receptor types are imbalanced for a prolonged time (Joels et al. 2008; de Kloet et al. 2007).

The secretion of cortisol and norepinephrine in response to acute stress is known to affect learning and memory (Smeets et al. 2011; van Stegeren et al. 2010). The mammalian brain does not house a solitary brain region mediating the acquisition, consolidation, and retrieval of all types of learned information. Instead, memory and learning are organized in multiple brain systems. Certain brain regions (e.g., the prefrontal cortex) govern goal-directed learning, whereas others (e.g., the dorsal striatum) are responsible for habit formation. Stress can induce a bias by promoting habit-based forms of learning and memory in lieu of goal-directed performance. Specifically, studies in rodents have determined that corticosterone and norepinephrine promote habit-based memory formation by acting on the amygdala, hippocampus, dorsal striatum, and prefrontal cortex—all of which also are involved in alcohol dependence. The relationship between cortisol and the vulnerability to alcohol dependence as well as to relapse after abstinence could involve cortisol's effects on habit-based learning. In view of the habit-like nature of addictive behaviors, it is fascinating that recent evidence indicates a role for the habit memory system located in the dorsal striatum in the maintenance and expression of drug-seeking and drug-taking behaviors (Everitt et al. 2008). For example, anxiety-inducing (i.e., anxiogenic) drugs can promote the use of dorsal striatal-dependent habit memory in rats (Packard 2009).

Research in humans also has shown that stress is associated with decreased use of cognitive behavioral strategies, which involve the hippocampus, and increased use of stimulus-response strategies, which involve the caudate nucleus (Kim et al. 2001; Schwabe et al. 2007). It is possible that the heightened cortisol responsivity in people at increased risk for alcohol dependence may promote the transition to heavy, hazardous drinking through cortisol's ability to promote habit-based memory formation and learning during alcohol intoxication, especially during states of heightened arousal (Smeets et al. 2009). Furthermore, the wide fluctuations in cortisol secretion observed in alcohol-dependent people could help maintain these habit-based addictive behaviors. Additionally, the hypercortisolism associated with alcohol dependence may in part promote relapse by favoring the use of habit-based memory to guide the expression of maladaptive behaviors. Finally, persistent hypercortisolism observed during repeated episodes of acute alcohol intoxication and withdrawal may be toxic to neurons in the hippocampus. Hippocampal damage, in turn, may result in alcohol-related symptoms such as personality changes, memory loss, and depression.

Chronic exposure to elevated glucocorticoid levels also can have a detrimental effect on prefrontal cortex function with concomitant neuronal degeneration (Bennett 2008). As mentioned earlier, the prefrontal cortex is involved in complex cognitive operations, including assessing likelihood of reward or punishment during critical decision-making situations as well as assessing internal and external affective cues and responding adaptively, particularly in stressful situations. Psychosocial stress can disrupt prefrontal cortex function in humans (e.g., Liston et al. 2009). However, the specific effects of glucocorticoids in this process remain to be determined (Het et al. 2005) because other physiological changes that occur as part of the overall stress response, such as increased catecholamine levels, also alter prefrontal cortex function (Qin et al. 2009). Animal studies have suggested that glucocorticoids play a role in the cognitive deficits observed after withdrawal from chronic alcohol consumption (Rose et al. 2010). In mice, the glucocorticoid receptor antagonist mifepristone reduced memory deficits during the first and second week after alcohol withdrawal, suggesting that heightened glucocorticoid levels during withdrawal directly contribute to these cognitive deficits (Jacquot et al. 2008). Studies in humans found that cognitive impairment in abstinent alcoholics was related to an attenuated cortisol response to a psychosocial stressor (Errico et al. 2002). Poorer cognitive performance also was related to more withdrawal episodes, heavier alcohol consumption, and higher cortisol levels during withdrawal (Errico et al 2002; Keedwell et al. 2001). Thus, further studies should investigate the mechanism through which altered stress regulation of the HPA axis impairs cognitive function and relates to poor prognosis in recovering alcoholics.

The amygdala is another limbic structure that is affected by cortisol in ways that might contribute to alcohol dependence. The amygdala is a major extrahypothalamic source of CRF-containing neurons that carry large numbers of CRF-1 and CRF-2 receptors; it has a primary role in the processing and memory of emotional reactions.

Thus, the extended amygdala is crucial for the expression of anxiety, and the central amygdala is a major extrahypothalamic site where CRF is produced and plays a role in mediating fear and anxiety (Gray and Bingaman 1996; Heilig et al. 1994). Whereas the hypothalamic CRF system is important for modulating neuroendocrine responses to stress, the extrahypothalamic CRF system manifests the behavioral response to stress via the amygdala and other limbic regions. In rats with high alcohol preference and anxiety levels, CRF gene expression is reduced in the central nucleus of the amygdala (Hwang et al. 2004); moreover, the extracellular levels of CRF in the central amygdala are increased during acute alcohol withdrawal and during exposure to various forms of stress (Merlo-Pich et al. 1995). Chronically elevated corticosterone levels also increase CRF expression in the central amygdala (Shepard et al. 2000; Schulkin et al. 1998). This enhanced CRF production may contribute to anxiety-like behaviors. The heightened or exaggerated emotional and fearful reactivity to perceived stress, in turn, may drive alcohol consumption observed during heavy, hazardous drinking and alcohol dependence. Consistent with this theory, administration of CRF antagonists reverses anxiety-like behaviors and excessive alcohol drinking associated with alcohol withdrawal (Valdez et al. 2003). These observations suggest that heightened cortisol exposure influences alcohol consumption by inducing anxiety and dysphoria via CRF-mediated activation of the amygdala.

Early Abstinence and Relapse

As mentioned earlier, a blunted hormonal response to stress during early abstinence is related to increased risk for relapse (Junghanns et al. 2003, 2005; Kiefer et al. 2002). The mechanism underlying this relationship is not clear. Because cortisol levels in alcohol-dependent people negatively correlate with self-reported alcohol craving (Bohn et al. 1995), it is possible that relapse to alcohol consumption during early abstinence partly is driven by alcohol's ability to induce cortisol elevation (Junghanns et al. 2005). If this is the case, cortisol may influence the motivation to drink and relapse via a potential negative-reinforcement pathway. Several observations support this hypothesis. For example, several studies evaluating pharmacological treatments for relapse prevention during early abstinence have examined the relationships among HPA activity, craving, and alcohol intake during early abstinence, based on the hypothesis that risk for relapse may be attenuated through mechanisms that reduce craving and increase cortisol. For example, O'Malley and colleagues (2002) administered naltrexone or placebo for 6 days to alcohol-dependent, non-treatment seekers who then participated in an alcohol self-administration session. Naltrexone treatment resulted in higher cortisol levels, which were associated with lower levels of craving and less alcohol consumption. Similarly, Kiefer and colleagues (2006) studied the efficacy of naltrexone and/or an agent that can block receptors for the neurotransmitter GABA (i.e., acamprosate), both of which are used in alcoholism treatment to reduce craving. The study found that without an active treatment, both ACTH and cortisol levels decreased during early abstinence; conversely, treatment with naltrexone and acamprosate prevented these declines. Moreover, increased ACTH and cortisol during treatment was associated with reduced risk of relapse. Finally, Sinha and colleagues (2009) found that alcohol-dependent patients who had been abstinent for 28 days showed significantly elevated basal cortisol levels as well as a blunted cortisol response to a psychological stressor and to exposure to an alcohol-related cue. Further, stress and cue exposure resulted in significantly enhanced and persistent craving. Although some studies have not been able to demonstrate correlations between changes in cortisol and craving (e.g., Pratt and Davidson 2009), decreased cortisol levels in general have been accompanied by increased craving during early abstinence, which may underlie risk for relapse to alcohol use. Taken together, these studies suggest that cortisol levels and HPA axis reactivity may be useful clinical indicators in the management of relapse risk and that manipulating HPA axis regulation through either pharmacological or psychosocial intervention is a viable avenue of research for developing new alcoholism treatments.

Summary

The HPA axis, an important physiological stress pathway, may play a significant role in the risk and development of AUDs, and the glucocorticoid cortisol may be useful as a biomarker for HPA axis homeostatic regulation. The hormones of the HPA axis act to maintain homeostasis in the presence of stress through a variety of mechanisms. When the HPA axis becomes dysregulated, regardless of cause, deviations in cortisol reactivity result that have been associated with the progressive stages of alcoholism risk, dependence, and abstinence (see figure 2). Considerable research has been devoted to identifying potential underlying mechanisms of the HPA axis dynamics that contribute to progressive stages of alcohol dependence, and the available evidence support several of these potential mechanisms.

First, non-alcohol–dependent drinkers believed to be at risk for developing an AUD, either because of their family history or because of their hazardous drinking patterns, clearly have altered HPA axis function compared with low-risk individuals. The findings regarding the exact nature of this dysregulation (i.e., whether the HPA axis shows hyper- or hyporesponsivity) are mixed, particularly within the family-history literature. However, the equivocal results most likely are related to differences in experimental strategies used and in the levels of alcohol consumption in these drinkers (e.g., tolerance level). Nevertheless, this body of literature generally has established that

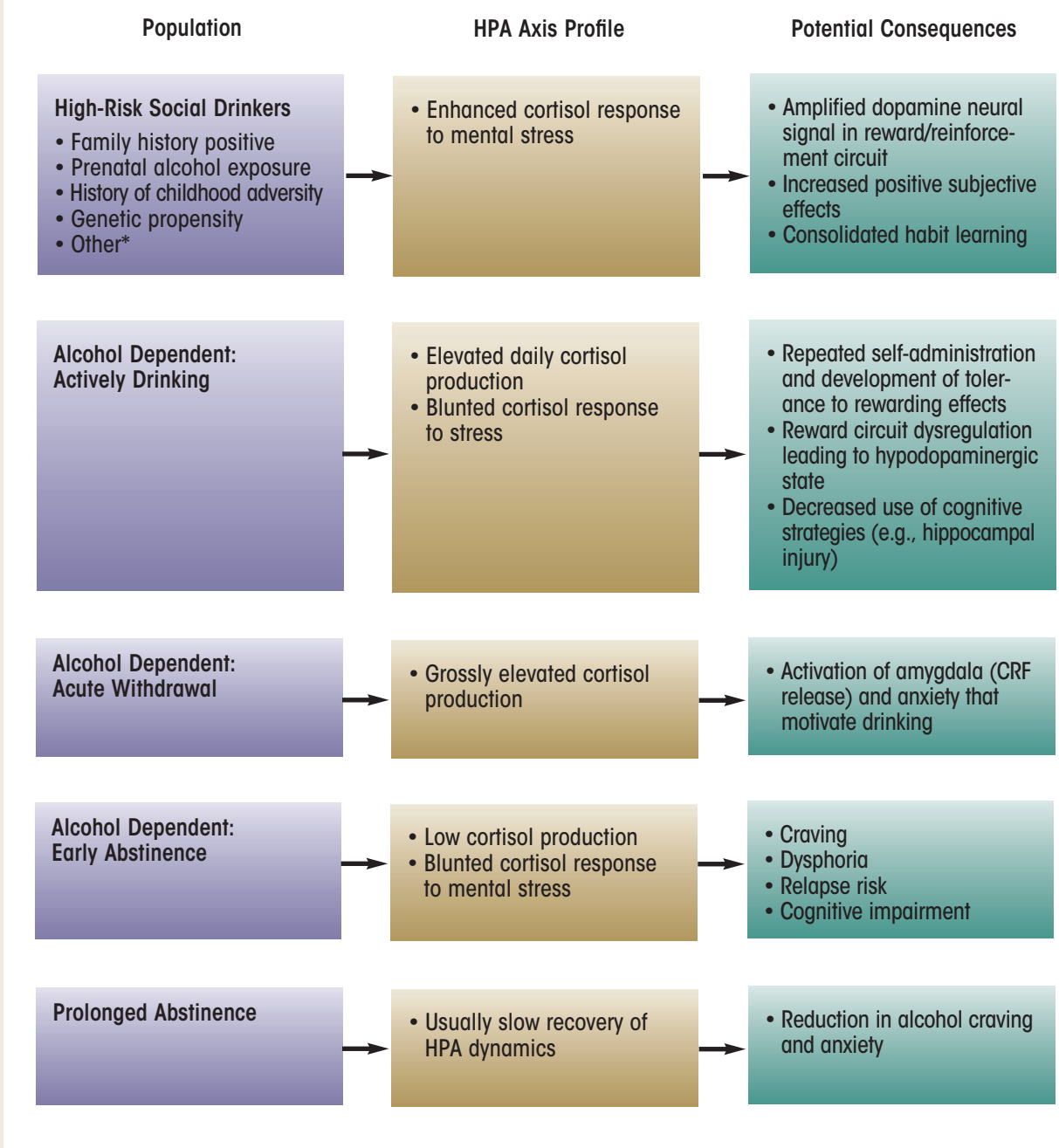

Figure 2 Summary of the activity of the hypothalamic–pituitary–adrenal (HPA) axis during different stages of alcoholism development and their potential consequences.

NOTE: *Low level of response (LR) to alcohol is a phenotype that predicts higher risk for alcohol-related problems (Hu et al. 2005); currently, there are no data characterizing HPA axis response to mental stress in this high-risk group. Posttraumatic stress disorder (PTSD) is a complicated disorder with multiple subtypes and comorbidities; the HPA axis profile of individuals with PTSD symptomatology generally is not thought to react to mental stress with enhanced responsivity and therefore does not fit the model depicted above for other high-risk social drinkers.

cortisol responsivity serves as a risk marker for the propensity for abuse or dependence.

Second, considerable evidence supports the effect of glucocorticoids in facilitating dopamine-mediated signal transmission in the brain, which has been linked to reward pathways involved in almost all drugs of abuse. Moreover, glucocorticoids themselves have positive reinforcing properties. Conversely, reduced glucocorticoid activity seems to suppress acquisition and self-administration of drugs of abuse (Fahlke et al. 1996; Goeders and Guerin 1996). Thus, glucocorticoids appear to play a critical mediating role in the dopamine reward circuit.

Third, cortisol plays a key role in brain regions that are important for cognitive learning and memory retrieval, encoding, and consolidation. These are central processes affected by shifting hyper- and hypocortisolism throughout alcohol dependence as well as by cortisol responses to stress. It is possible that such perturbations in the HPA axis consolidate the type of habit-based learning (rather than goal-directed learning) that sustains maladaptive behaviors related to alcohol use.

Finally, deficiency in cortisol response during early abstinence is predictive of relapse to alcohol and may modulate conditions that often accompany relapse episodes, such as craving, dysphoria, and severe withdrawal symptoms. Thus, cortisol levels during abstinence may be useful clinical indicators of relapse vulnerability, and interventions that increase cortisol and decrease craving might be useful to prevent relapse.

Taken together, HPA axis function may serve as a predictor of risk for alcohol dependence in alcohol-naïve or social drinkers, facilitate initiation and maintenance of alcohol use, or serve as a predictor for risk of relapse in abstinent alcohol-dependent individuals. Using HPA axis reactivity as a predictive marker may help to identify individuals at risk for dependence or relapse prior to development of those conditions, which would allow the individuals and their treatment providers to take action and improve overall prevention and treatment efforts for AUDs. ■

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Neural Pathways of Stress Integration

Relevance to Alcohol Abuse

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Stress is a critical component in the development, maintenance, and reinstatement of addictive behaviors, including alcohol use. This article reviews the current state of the literature on the brain's stress response, focusing on the hypothalamic–pituitary–adrenal (HPA) axis. Stress responses can occur as a reaction to physiological (or systemic) challenge or threat; signals from multiple parts of the brain send input to the paraventricular nucleus (PVN) within the hypothalamus. However, responses also occur to stressors that predict potential threats (psychogenic stressors). Psychogenic responses are mediated by a series of nerve cell connections in the limbic–PVN pathway, with amygdalar and infralimbic cortex circuits signaling excitation and prelimbic cortex and hippocampal neurons signaling stress inhibition. Limbic–PVN connections are relayed by predominantly GABAergic neurons in regions such as the bed nucleus of the stria terminalis and preoptic area. Chronic stress affects the structure and function of limbic stress circuitry and results in enhanced PVN excitability, although the exact mechanism is unknown. Of importance, acute and chronic alcohol exposure are known to affect both systemic and psychogenic stress pathways and may be linked to stress dysregulation by precipitating chronic stress–like changes in amygdalar and prefrontal components of the limbic stress control network.

KEY WORDS: Addiction; alcohol and other drug–seeking behavior; alcohol use and abuse; stress; stressor; chronic stress reaction; stress integration; physiological response to stress; psychogenic stress responses; brain; neural pathways; limbic-paraventricular pathway; limbic stress control network; hypothalamic–pituitary–adrenal axis; literature review

Adaptation in the face of physical or psychological adversity is required for the survival, health, and well-being of all organisms. Adverse events, often denoted as "stressors," initiate a diverse physiological response from multiple sources, including activation of the hypothalamic–pituitary–adrenal (HPA) axis.[1] The HPA axis is responsible for the glucocorticoid component of the stress response (i.e., steroid hormone response; cortisol in humans, corticosterone in mice and rats). Glucocorticoid secretion is thought to contribute to stress adaptation by causing long-term changes in gene expression via cognate adrenocorticosteroid receptors (i.e., mineralocorticoid receptor [MR] and glucocorticoid receptor [GR]). The adrenocorticosteroid receptors function as ligand-gated transcription factors (De Kloet et al. 1998) but can also modulate transcription by interfering with other transcriptional regulators, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) (Webster and Cidlowski 1999). Glucocorticoids also can have rapid effects on brain chemistry and behavior via nongenomic membrane signaling mechanisms (De Kloet et al. 2008). Glucocorticoids are thought to contribute to termination of the initial stress response (Keller-Wood and Dallman 1984) and to participate in long-term restoration of homeostasis triggered by the initial response (Munck et al. 1984).

Glucocorticoid stress responses can be initiated by physiological perturbations (representing reflexive responses) or by brain processes linking environmental cues with probable negative outcomes. The latter so-called "psychogenic" response is anticipatory in nature and involves brain pathways responsible for innate defense programs or memory of aversive events (Herman et al. 2003). Thus, the psychogenic response is related to prior experience, and it is designed to energetically prepare the organism to either avoid an adverse outcome or engage in behaviors that can maximize the potential for survival.

[1] For the definition of this and other technical terms, see the Glossary, pp. 522–524.

Considerable evidence indicates that stress systems play a major role in addictive processes, including alcohol dependence. For example, exposure to stress can precipitate relapse or increase alcohol use (Sinha 2007). Actions of stress/glucocorticoids on alcohol intake can be linked to modulation of reward/stress circuitry, including, for example, enhancement of dopamine release in the nucleus accumbens (Sutoo and Akiyama 2002; Yavich and Tiihonen 2000) and activation of central corticotropin-releasing factor (CRF) pathways (Heilig and Koob 2007). Notably, the link between alcohol intake and stress is complicated by the fact that exposure to alcohol, like many drugs of abuse, causes the release of glucocorticoids upon exposure and thus can be classified as an acute "stressor" of sorts (see Allen et al. 2011).

This article reviews the organization of neurocircuits that regulate stress responses, focusing on the HPA axis, which is of particular relevance to addictive processes (see Marinelli and Piazza 2002). It also discusses areas of intersection between stress and reward pathways, as these are likely important in mediating the deleterious effects of stress on substance abuse and addiction.

Circuitry Mediating the Reflexive Stress Response

The HPA axis is controlled by neurons within the paraventricular nucleus (PVN) in the hypothalamus (see figure 1). These neurons secrete CRF and the hormone vasopressin into the portal circulation, which then triggers the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary gland. ACTH travels via the systemic circulation to reach the adrenal cortex, wherein glucocorticoids are synthesized and released (see Herman et al. 2003).

Reflexive stress responses occur during emergencies (e.g., infection, starvation, dehydration, or shock), when the brain must respond to a substantial challenge to homeostasis by mobilizing the HPA axis. Sensory information is communicated to the PVN by first- or second-order neurons, generating a direct activation of CRF release (see Herman et al. 2003). For example, low blood pressure associated with blood loss is relayed via sensory nerves to brainstem neurons in the A2 catecholaminergic cell group (Palkovits and Zaborszky 1977), which then project directly to the PVN (Cunningham and Sawchenko 1988) and rapidly elicit noradrenergic activation of CRF neurons (Plotsky et al. 1989).

In addition to neural pathways, information on changes in physiological state also may be relayed via circulating factors that bind to areas outside the blood–brain barrier. For example, peripheral increases in the hormone angiotensin II (signaling dehydration) are sensed by receptors in the subfornical organ (which is located outside the blood–brain barrier and regulates fluid balance), which sends direct angiotensin II projections to the PVN CRF neurons, facilitating HPA activation (Plotsky et al. 1988). Some peripheral stimuli, such as inflammation, produce factors that can signal by multiple mechanisms; for example, the proinflammatory cytokine interleukin 1-b seems to activate the HPA axis via sensory nerve fibers in the vagus nerve; the area postrema, which is outside the blood–brain barrier; and perivascular cells in the region of the A2 cell group (Ericsson et al.

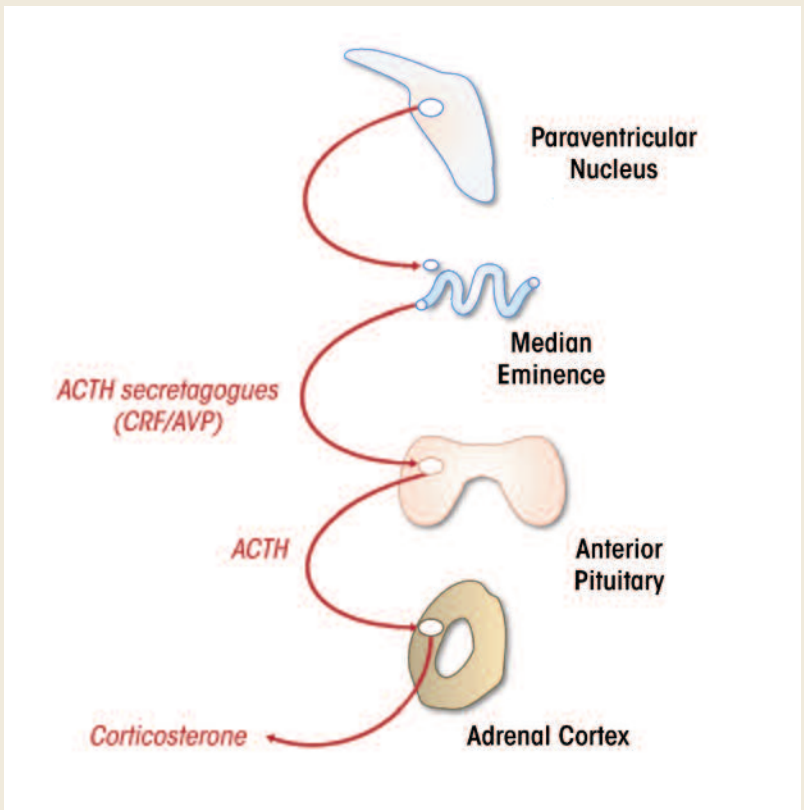

Figure 1 Schematic of the hypothalmic–pituitary–adrenal (HPA) axis of the rat. HPA responses are initiated by neurosecretory neurons of medial parvocellular paraventricular nucleus (mpPVN), which secretes adrenocorticotropin (ACTH) secretagogues such as corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) in the hypophysial portal circulation at the level of the median eminence. These secretagogues promote release of ACTH into the systemic circulation, whereby it promotes synthesis and release of glucocorticoids at the adrenal cortex.

1997; Lee et al. 1998; Wieczorek and Dunn 2006).

Drugs of abuse also may produce an initial corticosterone response via brainstem PVN-projecting pathways. For example, initial exposure to alcohol causes ACTH and corticosterone release, consistent with alcohol acting as an unconditioned stimulus (Allen et al. 2011). Acute HPA axis activation by alcohol is mediated by brainstem noradrenergic systems (Allen et al. 2011). However, chronic exposure to alcohol significantly blunts HPA axis activation to acute alcohol exposure (Rivier 1995), suggesting that, to some degree, direct HPA excitatory effects of alcohol use habituate over time.

Circuitry Subserving Anticipatory Stress Responses: The Limbic Stress-Control Network

Because true physiologic "emergencies" are relatively rare, the vast majority of stress responses are anticipatory in nature, involving interpretation of the threat potential of environmental stimuli with respect to previous experience or innate programs. Anticipatory stress responses are largely controlled by limbic forebrain structures, such as the hippocampus, medial prefrontal cortex (mPFC), and amygdala (see Ulrich-Lai and Herman 2009). These structures all receive processed sensory information and are involved in regulation of emotion, reward, and mood.

Brain lesion and stimulation studies indicate that the hippocampus inhibits the HPA axis. Electrical stimulation of the hippocampus decreases glucocorticoid release in rats and humans. Damage to the hippocampus, or the nerves carrying impulses away from it (i.e., lateral fornix), cause exaggerated responses to psychogenic stressors (e.g., restraint) and manifest as a prolonged return to baseline glucocorticoid levels (for primary references, see Herman et al. 2003; Jacobson and Sapolsky 1991). Some data suggest that the hippocampus also inhibits basal HPA axis activity, but this is not universally observed (Herman et al. 2003; Jacobson and Sapolsky 1991). The effects of hippocampal damage on psychogenic HPA axis stress responses can be localized to the ventral subiculum (vSUB), the main subcortical output of the ventral hippocampus (Herman et al. 2003). Discrete lesions of the vSUB in rats enhance PVN CRF peptide and mRNA expression and increase corticosterone release and PVN activation (as determined by induction of FOS mRNA expression) in response to restraint (Herman et al. 1998).

The effect of the vSUB on stress regulation is stressor specific. Lesions of the vSUB prolong HPA axis responses to novelty but do not affect reflexive responses (e.g., to ether inhalation) (Herman et al. 1998). Some evidence suggests that glucocorticoids play a role in hippocampal inhibition of anticipatory responses, as lesions can block feedback inhibition of the HPA axis by the synthetic steroid dexamethasone (Magarinos et al. 1987). In addition, mice with forebrain GR deletions, including the hippocampus, have exaggerated responses to restraint and novelty (but not hypoxia) and impaired dexamethasone suppression of corticosterone release (Boyle et al. 2005; Furay et al. 2008). Together, the data indicate that the hippocampus is specifically engaged in regulation of responses to psychogenic stressors, in keeping with its role in cognitive processing and emotion.

Unlike the hippocampus, the amygdala is associated with excitation of the HPA axis. Amygdalar stimulation promotes glucocorticoid release, whereas large lesions of the amygdaloid complex reduce HPA axis activity (see Herman et al. 2003). However, there is a marked subregional specialization of stress-integrative functions within the amygdala. The central nucleus of the amygdala (CeA) is highly responsive to homeostatic stressors, such as inflammation and blood loss (Dayas et al. 2001; Sawchenko et al. 2000). Lesions of the CeA attenuate HPA axis responses to these types of stimuli but not to restraint (Dayas et al. 1999; Prewitt and Herman 1997; Xu et al. 1999). In contrast, the medial nucleus of the amygdala (MeA) shows preferential FOS responses to stimuli, such as restraint (Dayas et al. 2001; Sawchenko et al. 2000). Lesions of the MeA reduce HPA axis responses to restraint and light and sound stimuli but not to systemic injection of the protein interleukin 1-b or ether inhalation (Dayas et al. 1999; Feldman et al. 1994). Thus, it seems that reflexive and anticipatory responses may be regulated in part by discrete amygdaloid circuitry.

The mPFC seems to have a complex role in stress regulation. All divisions of the rodent PFC are robustly activated by acute stress. However, the physiological consequences of stress activation seem to vary by region. The prelimbic division of the mPFC (PL) is important in stress inhibition because numerous studies have shown that damage to this region prolongs HPA axis responses to acute psychogenic (but not homeostatic) stressors (Diorio et al. 1993; Figueiredo et al. 2003; Radley et al. 2006), whereas stimulation inhibits stress responses (Jones et al. 2011). The mPFC seems to be a site for glucocorticoid feedback of HPA responses because local glucocorticoid implants inhibit anticipatory (but not reflexive) responses to stressors (Akana et al. 2001; Diorio et al. 1993). In contrast, lesions directed at the more ventral infralimbic PFC (IL) have a markedly different physiological effect. Damage to the IL decreases autonomic responses to psychogenic stressors (Tavares et al. 2009) and also attenuates PVN FOS activation in response to restraint (Radley et al. 2006). Thus, the PL and IL seem to have opposing effects on stress integration.

Running the Relay: Limbic–PVN Networks

Stimulation of the PVN by the hippocampus, prefrontal cortex, and amygdala is quite limited. Therefore, regulation of HPA axis output by these structures requires intermediary

synapses (see figure 2). Studies that trace projections from one part of the brain to another (i.e., tract-tracing studies) reveal the potential for bisynaptic limbic–PVN connections traversing a number of subcortical regions, including the bed nucleus of the stria terminalis (BNST), dorsomedial hypothalamus, medial preoptic area, and peri-PVN region (including the subparaventricular nucleus) (Cullinan et al. 1993; Prewitt and Herman 1998; Vertes 2004). Dual-tracing studies indicate that nerves carrying impulses away from the vSUB, MeA, and CeA (i.e., efferent nerves) directly contact PVN-projecting neurons in these regions, consistent with functional interconnections (Cullinan et al. 1993; Prewitt and Herman 1998).

The differential effects of PL and IL on stress effector systems may reflect their marked divergence in subcortical targets. The PL has substantial projections to reward-relevant pathways, including the nucleus accumbens and basolateral amygdala, as well as the posterior BNST, which is linked to HPA axis inhibition. In contrast, the IL has rich interconnections with regions involved in autonomic regulation, including the CeA, nucleus of the solitary tract (NTS), anteroventral BNST, and dorsomedial hypothalamus (Vertes 2004). Thus, it is probable that the net effect of PFC stress activation requires subcortical integration of PL and IL outflow.

Of note, mPFC, hippocampal, and amygdalar efferents tend to be concentrated in regions sending γ-aminobutyric acid (GABA)-carrying projections to the PVN (see figure 2). Indeed, the vast number of sub-innervated PVN-projecting neurons are GABAergic in phenotype. Projection neurons of the vSUB (as well as the mPFC) are glutamatergic in nature, thus suggesting that these cells engage in transsynaptic inhibition of the PVN following activation by stress. In contrast, the projection neurons of the MeA and CeA are predominantly GABAergic, suggesting that amygdalar excitation of the PVN is mediated by disinhibition, involving sequential GABA synapses (Herman et al. 2003).

The BNST is of particular interest, in that it receives inputs from all of the major limbic stress-integrative structures (CeA, MeA, vSUB, IL, and PL) (Cullinan et al. 1993; Dong et al. 2001; Vertes 2004). Of note, different BNST subregions seem to be responsible for inhibition versus excitation of HPA axis stress responses. For example, lesions of the posterior medial region of the BNST increase the magnitude of ACTH and corticosterone release and PVN FOS activation (Choi et al. 2007), implying a role in central integration of stress

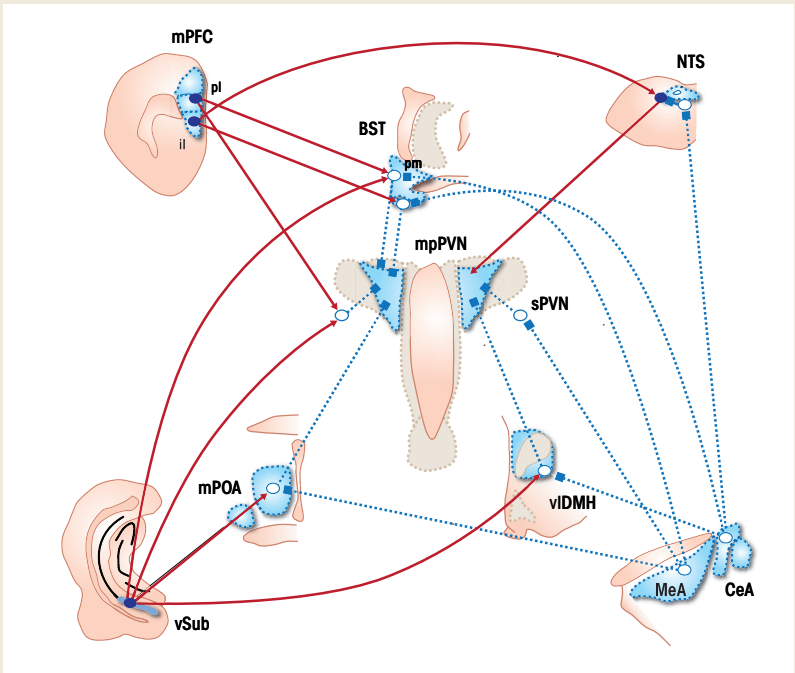

Figure 2 Schematic of limbic stress-integrative pathways from the prefrontal cortex, amygdala and hippocampus. The medial prefrontal cortex (mPFC) subsumes neurons of the prelimbic (pl) and infralimbic cortices (il), which appear to have different actions on the hypothalmic–pituitary–adrenal (HPA) axis stress response. The pl sends excitatory projections (designated as dark circles, filled line with arrows) to regions such as the peri-PVN (peri-paraventricular nucleus) zone and bed nucleus of the stria terminalis (BNST), both of which send direct GABAergic projections to the medial parvocellular PVN (delineated as open circles, dotted lines ending in squares). This two-neuron chain is likely to be inhibitory in nature. In contrast, the infralimbic cortex projects to regions such as the nucleus of the solitary tract (NTS) and the anterior BNST, which sends excitatory projections to the PVN, implying a means of PVN excitation from this cortical region. The ventral subiculum (vSUB) sends excitatory projections to numerous subcortical regions, including the posterior BNST, peri-PVN region (including the subparaventricular zone [sPVN], medial preoptic area [POA] and ventrolateral region of the dorsomedial hypothalamic nucleus [vlDMH]), all of which send GABAergic projections to the PVN and are likely to communicate transsynaptic inhibition. The medial amygdaloid nucleus (MeA) sends inhibitory projections to GABAergic PVN-projecting populations, such as the BNST, POA and sPVN, eliciting a transsynaptic disinhibition. A similar arrangement likely exists for the central amygdaloid nucleus (CeA), which sends GABAergic outflow to the ventrolateral BST and to a lesser extent, the vlDMH. The CeA also projects to GABAergic neurons in the NTS, which may disinhibit ascending projections to the PVN.

inhibition. Lesions of the anteroventral component of the BNST also enhance stress responses (Radley et al. 2009). In contrast, larger lesions of the anterior BNST reduce HPA axis stress responses (Choi et al. 2007), consistent with a role for this region in stress excitation. Thus, the role of the BNST in stress inhibition versus activation is compartmentalized and may be associated with differences in limbic targeting of individual subregions of the BNST. For example, the posterior medial BNST receives heavy innervation from the vSUB and MeA, whereas the anteroventral region receives input from the CeA and most of the IL efferents (Canteras and Swanson 1992; Cullinan et al. 1993; Dong et al. 2001; Vertes 2004).

The medial preoptic area and peri-PVN regions are heavily populated with GABAergic neurons and seem to primarily modulate stress inhibition (Herman et al. 2003). Neurons in these regions are believed to provide tonic inhibition to the PVN, which can be adjusted in accordance with glutamate inputs from the vSUB (enhanced inhibition) or GABAergic inputs primarily from the MeA (disinhibition). Lesions of the medial preoptic nucleus increase HPA axis stress responses and block HPA axis responses elicited by medial amygdalar stimulation, suggesting a primary role in stress inhibition (for primary references, see Herman et al. 2003). Local inhibition of glutamate signaling in the peri-PVN region also enhances HPA axis stress responses (Ziegler and Herman 2000), suggesting that limbic axons terminating in this region may modulate PVN activation.

It is more difficult to pinpoint the role of other hypothalamic regions linking limbic efferents to the PVN, such as the dorsomedial nucleus (Herman et al. 2003). For example, conflicting results are observed following lesion, activation, or inactivation of this dorsomedial hypothalamus, possibly because of heavy mixing of glutamate and GABA neuronal populations (Herman et al. 2003).

Additional potential relays remain to be fully explored. For example, the raphe nuclei and NTS innervate the PVN, are targeted by limbic structures (such as the PL) (see Vertes 2004) and are involved in stress excitation by serotonin and norepinephrine (Herman et al. 2003), respectively. However, as yet, there are no anatomical studies describing bisynaptic limbic–PVN relays through these regions.

Circuitry Subserving Chronic Stress Responses

Prolonged or extended exposure to stress causes long-term upregulation of the HPA axis, characterized by reduced thymus weight (attributed to cumulative elevations in GCs); increased adrenal size (attributed to increased ACTH release); increased adrenal sensitivity to ACTH; facilitated HPA axis responses to novel stressors; and in some (but not all) paradigms/conditions, elevated basal GC secretion (see Herman et al. 1995; Ulrich-Lai et al. 2006). Changes in peripheral hormone release are accompanied by increased PVN CRF and vasopressin mRNA (Herman et al. 1995), suggesting that HPA upregulation is centrally mediated. In addition, chronic stress increases glutamatergic and noradrenergic terminal abutting PVN CRF neuronal somata and dendrites, consistent with enhanced excitatory synaptic drive (Flak et al. 2009).

Central mechanisms of chronic HPA axis activation have yet to be determined. The role of the limbic forebrain in stress control suggests that differential involvement of the PFC, hippocampus, and amygdala may be responsible for prolonged drive. Of note, all regions show significant chronic stress–induced neuroplastic changes: Dendritic retraction is evident in hippocampal and mPFC pyramidal neurons, whereas dendritic extension is observed in the amygdala (for primary references, see Ulrich-Lai and Herman 2009). These studies are consistent with redistribution of limbic input to HPA excitatory circuits, favoring excitation over inhibition.

Enhanced amygdalar drive is proposed to play a major role in chronic stress pathology. For example, chronic stress activates the CeA CRF system, which has been proposed as a chronic stress–recruited pathway (Dallman et al. 2003). However, the CeA does not seem to be required for the development or maintenance of chronic stress symptoms (Solomon et al. 2010). In addition, lesions of the MeA also fail to prevent chronic stress drive of the HPA axis (Solomon et al. 2010). Thus, the overall link between amygdalar hyperactivity and chronic stress–induced HPA axis dysfunction has yet to be firmly established.

The paraventricular nucleus of the hypothalamus (PVT) seems to comprise a component of the chronic-stress pathway. Lesions of the PVT block chronic stress sensitization of HPA axis responses to novel stressors (Bhatnagar and Dallman 1998), suggesting a primary role in the facilitation process. In addition, PVT lesions disrupt the process of HPA axis habituation to repeated stressors (Bhatnagar et al. 2002). Taken together, the data suggest the PVT plays a major role in gating HPA axis drive in the context of prolonged stress exposure. Of note, the PVT and limbic forebrain sites that control acute stress responses are interconnected (see Vertes and Hoover 2008), allowing for possible coordination of corticolimbic stress outputs in this region. The PVT also is positioned to process information regarding ongoing physiological status, receiving inputs from orexinergic neurons (which regulate the release of acetylcholine, serotonin, and noradrenaline) of the dorsolateral hypothalamus (which plays an integral role in control of arousal processes) and ascending brainstem systems involved in autonomic control.

The BNST also is positioned to integrate information on chronic stress. Lesions of the anteroventral BNST attenuate responses to acute stress, but potentiate facilitation of the HPA axis by chronic stress (Choi et al. 2008). These data suggest that this region has chronicity-dependent roles in HPA axis control, with presumably different

neural populations recruited in an attempt to respond to prolonged stress exposure. Given intimate interconnectivity between the anterior BNST and mPFC, hippocampus, and amygdala, it is possible that BNST neurons may be "reprogrammed" by chronic stress–induced changes in limbic activity or innervation patterns.

Stress Circuitry and Alcohol

Readers familiar with the alcohol literature will no doubt find considerable overlap between the stress circuitry described above and brain circuitry linked to alcohol intake. For example, considerable data support a role for the CeA, BNST, and noradrenergic systems in the maintenance of alcohol dependence (see Koob 2009), suggesting that the process of addiction is linked to activation of stress (and HPA axis) excitatory pathways. Indeed, enhanced CeA/BNST CRF expression resembles what would be expected after chronic stress, leading to the hypothesis that negative addictive states (e.g., avoidance of withdrawal) are linked to alcohol-induced recruitment of chronic stress circuits (Koob 2009). Conversely, activation of reward pathways is known to significantly buffer stress reactivity via the amygdaloid complex, suggesting a mechanism whereby the rewarding effects of alcohol may reduce perceived stress (Ulrich-Lai et al. 2010).

Alcohol also has profound effects on medial prefrontal cortical neural activity, and chronic use is associated with prefrontal hypofunction (poor impulse control) in humans (see Abernathy et al. 2010). The mPFC projects to both the CeA and BNST and, at least in the case of the prelimbic region, plays a prominent role in HPA inhibition. In combination with the gain of function seen in amygdalar–BNST circuits, these observations suggest that chronic alcohol use causes marked changes across the limbic stress control network, biasing the organism for stress hyperreactivity.

Overall, adequate control of the HPA axis is a requirement for both short- and long-term survival. Given that key control nodes of HPA axis activity are targeted by alcohol, and that alcohol itself constitutes a threat, it is not surprising that corticosteroids, the "business end" of the axis, have profound interactions with both behavioral and physiological regulation of intake. The overlap between HPA regulatory and addiction circuits identifies key points that may be targets for both the long-term detrimental effects of alcohol abuse as well as dependence itself. The importance of circuit overlap is further underscored by the powerful reciprocal relationship between life stress and drinking, which complicates efforts to establish and maintain abstinence. ■

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Circadian Genes, the Stress Axis, and Alcoholism

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The body's internal system to control the daily rhythm of the body's functions (i.e., the circadian system), the body's stress response, and the body's neurobiology are highly interconnected. Thus, the rhythm of the circadian system impacts alcohol use patterns; at the same time, alcohol drinking also can alter circadian functions. The sensitivity of the circadian system to alcohol may result from alcohol's effects on the expression of several of the clock genes that regulate circadian function. The stress response system involves the hypothalamus and pituitary gland in the brain and the adrenal glands, as well as the hormones they secrete, including corticotrophin-releasing hormone, adrenocorticotrophic hormone, and glucocorticoids. It is controlled by brain-signaling molecules, including endogenous opioids such as β-endorphin. Alcohol consumption influences the activity of this system and vice versa. Finally, interactions exist between the circadian system, the hypothalamic–pituitary–adrenal axis, and alcohol consumption. Thus, it seems that certain clock genes may control functions of the stress response system and that these interactions are affected by alcohol. **KEY WORDS: Alcohol consumption; alcohol use, abuse and dependence; alcohol and other drug use pattern; genetics; genetic factors; circadian system; clock genes; stress; stress response; biological adaptation to stress; neurobiology; hypothalamic– pituitary–adrenal axis**

Alcohol abuse and dependence are estimated to affect 1 in 8 adults in the United States and several hundred million people worldwide (Grant et al. 2004). To define at-risk populations and develop better treatments, it is important to further identify the genetic and environmental factors that contribute to alcohol addiction. Recent evidence suggests that the body's internal system that helps control the daily rhythm of the body's activities (i.e., the circadian system), the body's stress response system, and the body's neurobiology of alcohol are extensively intertwined. This article explores some of these interactions.

The Circadian System and Alcohol's Effects on It

The circadian system—or the body's internal clock—is a naturally present regulatory system that helps the body maintain an approximately 24-hour cycle in biochemical, physiological, or behavioral processes, thereby allowing the organism to anticipate and prepare for regular environmental changes (i.e., the day–night cycle). For example, circadian rhythms maintain not only sleeping and feeding patterns but also physiological processes such as body temperature, brainwave activity, hormone production, and cell regeneration. The circadian clockwork results from the interaction of specific clock genes, including genes known as *Period* (*Per1, Per2,* and *Per3*), *Clock*, *Bmal1*, and *Cryptochrome* (*Cry1* and *Cry2*), and others.[1] The activity of these genes is controlled by two tightly coupled transcriptional and translational feedback loops that sustain a near 24-hour periodicity of cellular activity. Expression of these clock genes, in turn, regulates the expression of other clock-controlled genes (Ko and Takahashi 2006).

In both humans and animal models, complex bidirectional relationships seem to exist between alcohol intake or exposure and circadian clock systems. The impact of the circadian system on alcohol use is shown by the fact that both preference for and consumption of alcohol are modulated by time of day, and studies found that genetic interactions link core circadian clock genes with alcohol drinking (Spanagel et al. 2005*a, b*). In addition, disruption of the normal circadian rhythm (i.e., circadian desynchronization) seems to increase the use of alcohol, as seen in frequent travelers and rotating-shift workers, possibly because it frequently activates the body's stress response (i.e., increases the allostatic load[2]) (Rosenwasser et al. 2010; Trinkoff and Storr 1998). At the same time, a strong relationship seems to exist between alcohol drinking and altered circadian functions. For example, alcohol intake can alter the following circadian responses:

- Circadian rhythms in blood pressure, core body temperature, and hormone release in humans (Danel et al. 2009; Devaney et al. 2003; Nakashita et al. 2009);
- Shifts in the normal circadian rhythm (i.e., circadian phase shifting) and in the free-running period[3] in mice (Prosser et al. 2008; Seggio et al. 2009);
- Return to a normal circadian rhythm after a disruption (i.e., circadian phase resetting) and nocturnal activity patterns in hamsters (Ruby et al. 2009; Seggio et al. 2007); and

[1] By convention, gene names in animals are written in uppercase and lowercase and italicized. Gene names in humans are written in all caps and are italicized, whereas the acronyms for the encoded proteins are all caps but not italicized.

[2] The term allostatic load refers to the physiological consequences of chronic exposure to fluctuating or heightened hormonal responses resulting from repeated or chronic stress.

[3] Free-running period is a period that is not adjusted or entrained to the 24-hour cycle in nature or to any artificial cycle.

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- Rhythmicity in the activity of certain brain cells (i.e., proopiomelanocortin [POMC][4]-producing neurons) in a brain region called the hypothalamus (which is involved in the body's stress system) in rats (Chen et al. 2004).

Even alcohol exposure before birth can interfere with circadian systems. Thus, prenatal ethanol exposure in rats can alter core body temperature and phase-shifting ability (Sakata-Haga et al. 2006); rhythmic activity of the pituitary gland and the adrenal gland, both of which are part of the body's stress response system (Taylor et al. 1982); the rhythmic release of the main stress hormone (i.e., corticosterone) (Handa et al. 2006); immune cell rhythms (Arjona et al. 2006); and circadian expression of POMC in the hypothalamus (Chen et al. 2006).

Why Is the Body's Circadian System So Vulnerable to Alcohol Toxicity?

One logical explanation for the sensitivity of the circadian system to alcohol suggests that alcohol specifically targets one or more of the genes that regulate circadian functions. Using different experimental designs, researchers have demonstrated that alcohol exposure significantly alters the expression of several core clock genes. For example, in chronic alcohol-drinking rats, circadian expression of *Per1* and *Per2* is significantly disrupted in the hypothalamus (Chen et al. 2006). Likewise, prenatal alcohol exposure alters circadian expression of *Per1* and *Per2* genes in the hypothalamus and in tissues in other parts of the body in rats and mice (Arjona et al. 2006; Chen et al. 2004; Ko and Takahashi 2006). In addition, neonatal alcohol exposure reduces *Cry1* expression in a brain region called the suprachiasmatic nucleus and advances the phase of the *Per2* rhythm in the cerebellum and liver (Farnell et al. 2008). In human studies, the expression of clock genes (*PER*, *CRY,* and *BMAL1*) is reduced in white blood cells of male alcoholic patients (i.e., after chronic alcohol exposure) (Huang et al. 2010), whereas alcohol drinking in healthy males (i.e., acute exposure) increases *BMAL1* expression in these cells (Ando et al. 2010). Finally, variations of the *PER2* gene in which individual DNA building blocks are altered (i.e., single nucleotide polymorphisms [SNPs]) are associated with increased alcohol consumptions in male patients (Spanagel 2005*a*) and adolescent boys (Comasco et al. 2010). These observations suggest that clock genes are targets through which alcohol may alter circadian functions. However, in-depth molecular studies are necessary to elucidate the potential mechanisms by which alcohol directly or indirectly affects clock gene expression and cellular functions.

[4] POMC is a precursor molecule primarily produced in and secreted by the pituitary gland but also in the hypothalamus. POMC subsequently can be processed in other tissues into numerous different products, which in turn exert specific effects on the organism and play a role in a wide range of physiological processes. One of these products is adrenocorticotropic hormone (ACTH), which is produced in the pituitary gland and is part of the body's stress response system, the hypothalamic–pituitary–adrenal (HPA) axis.

Circadian Systems, the Stress Response, and Alcohol Consumption

The Stress Response System

The circadian system also may be involved in regulating alcohol-drinking behavior by interacting with a hormone system called the hypothalamic–pituitary–adrenal (HPA) axis, which plays a central role in the body's stress response as well as in reward mechanisms. Stress increases the production of a hormone called corticotrophin-releasing hormone (CRH) in certain cells in a region known as the paraventricular nucleus (PVN) in the hypothalamus. The CRH then is secreted into the blood vessels leading to the pituitary gland, where it interacts with a specific molecule, the CRH receptor1 (CRHR1), on specific cells in the anterior pituitary. In response, these cells begin the synthesis and release of adrenocorticotropic hormone (ACTH) into the circulation. ACTH, in turn, stimulates the release of glucocorticoids (i.e., corticosterone in rats and cortisol in humans) from the outer layer (i.e., cortex) of the adrenal glands that are located on top of the kidneys. The glucocorticoids then act on numerous tissues throughout the organism to coordinate the body's stress response. However, the CRH/CRHR1 system is found not only in the hypothalamus but also in other areas of the brain and helps mediate the actions of the brain's central stress response systems.

The CRH–HPA system is controlled by many brain-signaling molecules (i.e., neurotransmitters) and their receptors, including opioid peptides[5] (e.g., β-endorphin [β-EP]) and their receptors. For example, in rats, the bodies of CRF-producing cells are found in the same locations of the PNV as the fibers of β-EP–releasing cells. In another area of the hypothalamus called the median eminence, a certain type of opioid receptors (i.e., μ-opioid receptors [MOP-r]) is located on the ends of CRH-releasing cells. Agents that stimulate the activity of this receptor (i.e., MOP-r agonists) can inhibit neurotransmitter-stimulated CRF release from the hypothalamus in vitro. Likewise, studies in living organisms found that β-EP infusion decreased CRH release in the blood vessels linking the hypothalamus and the pituitary (Plotsky 1991), and morphine pretreatment prevented stress-induced HPA activation (Zhou et al. 1999). Finally, transplantation of β-EP–producing cells into the PVN suppressed HPA activation under different conditions and normalized stress hyperresponse in fetal alcohol-exposed rats (Boyadjieva et al. 2009). All of these data suggest that endogenous opioids (and, by extension, opiate drugs) have a counterregulatory effect on the stress response.

Alcohol and the Stress Response

In the central nervous system, β-EP long has been suspected of contributing to the positive reinforcement and motivational

[5] Opioid peptides are short sequences of amino acids (i.e., peptides) that are naturally produced by the body and have effects resembling those of opiate drugs. The three main classes of endogenous opioids are endorphins, enkephalins, and dynorphins. Endorphins also are derived from POMC, which also is the precursor for ACTH.

properties of several addictive substances. For example, microinjection of this peptide to several regions of the brain's reward system that involves the neurotransmitter dopamine (i.e., the mesolimbic dopamine system), such as the nucleus accumbens, produced place preference (Bals-Kubik et al. 1993). In addition, several studies have demonstrated that repeated administration of alcohol, cocaine, or heroin significantly attenuated β-EP expression in various limbic areas (Jarjour et al. 2009; Rasmussen et al. 2002; Sweep et al. 1988), supporting the notion that β-EP may contribute significantly in the development of alcohol abuse and dependence.

The stress response system also interacts with these reward pathways. For example, the CRH/CRHR1 system can activate mesolimbic dopaminergic pathways and increase dopamine-mediated signal transmission in various parts of the mesolimbic system, including the nucleus accumbens, amygdala, and medial prefrontal cortex. Furthermore, elevation of plasma corticosterone has been associated with increases in alcohol self-administration (Fahlke et al. 1995). Finally, evidence indicates that corticosterone directly stimulates activity of the mesolimbic dopamine system, subsequently increasing drug-seeking behavior (Piazza et al. 1996). Thus, stress, via activation of the CRH–HPA circuits and/or extrahypothalamic CRH circuits, increases mesolimbic dopamine that, in turn, increases drug seeking in drug-treated animals. The relationship between the stress response and the mesolimbic dopamine system is further supported by findings that an abnormality in POMC-mediated regulation of the HPA axis may lead to excess alcohol drinking under stressful conditions. Finally, consistent with animal studies demonstrating acute and chronic effects of alcohol on the HPA axis (Koob and Bloos 1998), studies in humans have documented HPA axis alterations in both actively drinking and recently abstinent alcoholics (Sinha 2007; Uhart and Wand 2009).

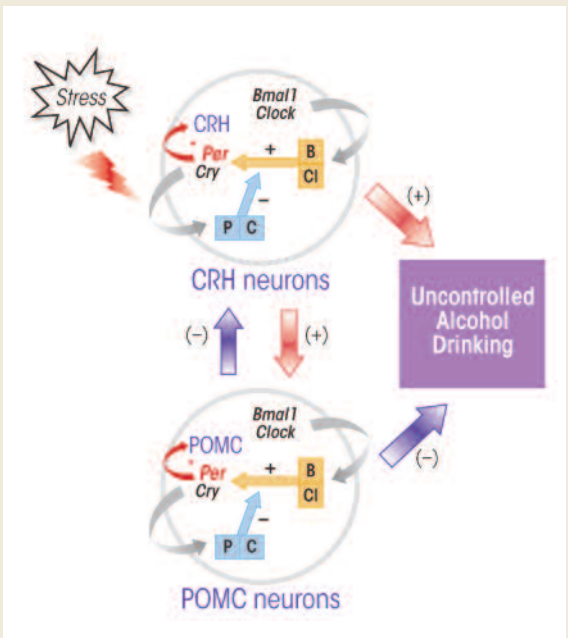

Figure Conceptual framework of how the circadian genes regulating stress-induced excess alcohol drinking. Clock genes (*Per* = P, *Cry* = C, *Bmal1* = B, and *Clock* = Cl) are key components of the circadian mechanism controlling the functions of nerve cells in the hypothalamus and pituitary that produce two molecules important in the body's stress response—corticotrophin-releasing hormone (CRH) and proopiomelanocortin (POMC). Of these clock genes, *Per* might be a potential target of alcohol (indicated by a * symbol) in CRH and POMC neurons and may control the stress-induced propensity to consume alcohol.

NOTE: (+) = stimulatory effect; (–) = inhibitory effect.

Circadian Genes, the Stress Response, and Alcohol

Several findings have suggested that interactions exist between the circadian system, the HPA axis, and alcohol-drinking behavior (see the figure). For example, in animal studies, forced-swimming and immobilization stress elevated expression of the murine *Per1* gene in CRH-positive cells of the PVN (Takahashi et al. 2001). On the other hand, stress-related (i.e., cortisol-induced) transcriptional activation of human *PER1* was reduced in a type of human blood cells (i.e., B-lymphoblastoid cells) that carried an altered form of the *PER1* gene (i.e., the rs3027172 genotype), which has been associated with an increased risk of alcoholism (Dong et al. 2011). Moreover, alcohol consumption can decrease *Per2* expression in POMC-producing neurons in the hypothalamus (Chen et al. 2004), and certain mutations in the murine *Per2* gene interfere with alcohol's stimulatory effect on POMC neurons (Agapito et al. 2010) and alter the rhythmic changes in corticosterone levels in the blood (Yang et al. 2009). Thus, it seems that the *Per1* and *Per2* genes may control functions of CRH- and POMC-producing neurons and that these interactions are affected by alcohol.

It is possible that alcohol-mediated modulation of *Per* genes may play a significant role in modulating HPA axis function, which in turn may lead to an increased propensity to drink alcohol following a stressful event. This view is supported by the recent findings by Dong and colleagues (2011) that the presence of certain *Per1* mutations increased psychosocial stress-induced alcohol drinking in mice, increased alcohol-drinking behavior in human adolescents following psychosocial adversity, and reduced cortisol-induced transcriptional activation of *Per1* in human B-lymphoblastoid cells. Other recent findings, although preliminary, showed that a certain *Per2* mutation increased basal levels of plasma corticosterone and alcohol drinking while preventing stress-induced increases in corticosterone levels and alcohol drinking in mice (Logan et al. 2011). In this context, it is interesting to note that mice carrying mutations in *Per2*, but not *Per1*, display ethanol reinforcement and alcohol-seeking behavior (Spanagel et al. 2005*a*; Zghoul et al. 2007).

Conclusions

The studies reviewed here suggest an intricate interaction between circadian genes, the body's stress response, and alcohol consumption. Thus, it seems that particularly the *Per1* and *Per2* genes, which have a distinct influence on the HPA axis, may control stress-induced propensity to alcohol drinking behavior. However, additional research is needed to address this novel concept involving clock genes, stress, and alcohol drinking. ■

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Immune Function Genes, Genetics, and the Neurobiology of Addiction

Fulton T. Crews, Ph.D.

The neuroimmune system (i.e., the immune system and those components of the nervous system that help regulate immune responses), and in particular the innate immune system, play a role in the development of addictions, including alcoholism, particularly in the context of stressful situations. Certain cells of the neuroimmune system are activated both by stress and by environmental factors such as alcohol, resulting in the induction of genes involved in innate immunity. One of the molecules mediating this gene induction is a regulatory protein called nuclear factor-κB, which activates many innate immune genes. Innate immune gene induction in certain brain regions (e.g., the frontal cortex), in turn, can disrupt decision making, which is a characteristic of addiction to alcohol and other drugs. Likewise, altered neuroimmune signaling processes are linked to alcohol-induced negative affect and depression-like behaviors and also regulate alcohol-drinking behavior. Moreover, the expression of several genes and proteins involved in innate immunity is enhanced in addicted people. Finally, specific variants of multiple innate immune genes are associated with the genetic risk for alcoholism in humans, further strengthening the connection between increased brain innate immune gene expression and alcohol addiction. KEY WORDS: Other drug dependence; alcoholism; addiction; causes of alcohol and other drug use; genetic factors; environmental factors; neurobiology; neuroimmune system; immune system; innate immune system; innate immune genes; immune function genes; nuclear factor-κB; stress; decision making; depression

The nervous system and the immune system interact closely to regulate the body's immune responses, including inflammatory responses. Accordingly, the term "neuroimmune system" refers to the immune system and those components of the nervous system that help regulate immune responses and also encompasses the hormones and other signaling molecules that convey signals between the immune and nervous systems. Part of the neuroimmune system is the innate immune system—a network of cells and the signaling molecules they release that are present from birth and form the first line of the body's defense system, including such responses as inflammatory reactions. This article summarizes the role that the neuroimmune system and genes encoding components of the innate immune system play in the development of addiction, including alcoholism.

Neuroimmune Signaling, Drug Abuse, and Stress

Neuroimmune signaling influences the responses and functions of a variety of body systems, including the digestive (i.e., enteric) system, sensory pathways, and the hormonal axis known as the hypothalamic–pituitary–adrenal (HPA) axis, which is involved in the body's stress response and also plays a role in addiction to alcohol and other drugs (AODs).[1] Immune cells called monocytes and monocyte-like cells in the brain (e.g., microglia) are sensitive key cells involved in neuroimmune signaling. When the immune system is stimulated or tissue damage occurs, these cells go through multiple stages of activation, which at the molecular level are reflected by the activation of a cascade of innate immune genes (Graeber 2010). These responses of the monocytes and microglia involve the production and secretion of signaling molecules, including inflammation-promoting (i.e., proinflammatory) cytokines and chemokines, such as monocyte chemotactic protein (MCP)-1, tumor necrosis factor α (TNFα), and interleukin 1β (IL1β). In the brain, microglial activation contributes to the activation of another type of cell called astroglia, or astrocytes, which, like microglia, show multiple stages of neuroimmune activation. In the microglia, the different stages of activation are accompanied by morphological changes. Thus, these cells change from their resting state with multiple branches (i.e., the ramified form) to a less branched, bushy morphology after mild activation and a rounded morphology after strong activation (i.e., when major brain cell death occurs). Chronic alcohol treatment induces mild, bushy microglial activation as well as mild astrocyte activation (see figure 1).

Activated glia show increased production of a wide range of proteins. For example, they produce and secrete increased amounts of proteases as well as of proteins found in the space between cells (i.e., extracellular matrix proteins). In addition, they generate increased amounts of proteins called toll-like receptors (TLRs) that play a role in alcohol-induced depressed mood and negative emotions (see below) and

[1] Among the main molecules involved in the HPA system are the glucocorticoids (e.g., cortisol), and cycles of stress as well as AOD abuse lead to elevated basal glucocorticoid levels and promote addiction (Armario 2010).

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show enhanced activity of enzymes known as oxidases that promote inflammatory reactions (e.g., nicotinamide adenine dinucleotide phosphate [NADPH] oxidases [NOX], cyclooxygenase [COX], and inducible nitric oxide synthases [iNOS]).

Microglia easily can become activated, and the initial stages of activation are characterized by the secretion of signaling molecules, slight morphological changes, and increased production of molecules involved in immune responses (i.e., major histocompatibility complex [MHC]) as well as of TLRs (Graeber 2010). Activation of microglia and astrocytes also increases proinflammatory agents, including TNFα, that alter the transmission of nerve signals (i.e., neurotransmission), including signal transmission mediated by the excitatory neurotransmitter glutamate. Likewise, studies have suggested that alcoholism is related to excessive glutamate levels (i.e., a hyperglutamate state). In the outer layer of the brain (i.e., the cerebral cortex), chronic alcohol-induced neuroimmune activation leads to a hyperglutamate state that reduces cortical function (figure 2). One mechanism contributing to this hyperglutamate state involves TNFα, which acts to reduce the activity of glutamate transporters[2] in the astrocytes (Zou and Crews 2005). Similarly, beverage alcohol (i.e., ethanol) has been shown to inhibit glutamate transport (Zou and Crews 2006). This blockade of glutamate transporters increases glutamate levels outside the cells and particularly in the space between two neurons where nerve signals are transmitted (i.e., the synapse), resulting in excessive neuronal activity (i.e., hyperexcitability). TNFα also stimulates the production of certain proteins found on signal-receiving neurons that interact with glutamate (i.e., the AMPA glutamate receptors) (Beattie et al. 2010). Increases in synaptic glutamate receptors and glutamate concentrations cause hyperexcitability that disrupts the normal concentration of the brain's response to a specific area of the cortex (i.e., cortical focus), thereby reducing cortical function. Through these mechanisms, monocytes, microglia, and astrocytes progressively become activated by stress and environmental factors, including ethanol, resulting in the induction of genes that encode proteins involved in the innate immune response.

Stress and Drug Abuse Increase Transcription of Innate Immune Genes

Stress and AODs, as well as sensory and hormonal signals, activate a regulatory protein (i.e., transcription factor[3]) called nuclear factor κ–light-chain enhancer of activated B cells (NF-κB) that is produced in large amounts (i.e., is highly expressed) in monocytes and microglia. Although NF-κB is found in most cells, it is the key transcription factor involved in the induction of innate immune genes in microglia and other monocyte-like cells. A wide range of stimuli, such as stress, cytokines, oxidative free radicals, ultraviolet irradiation, bacterial or viral molecules, and many other signaling molecules, increase binding of NF-κB to specific sequences of the DNA. This binding increases the transcription of many genes, particularly those encoding signaling molecules (e.g., chemokines and cytokines) and enzymes (e.g., oxidases and proteases) (figure 3). Studies found that ethanol can increase the binding of NF-κB to its corresponding DNA sequences both in the brains of living organisms (Crews et al. 2006) and in cultured brain slices obtained from a brain area called the hippocampal–entorhinal

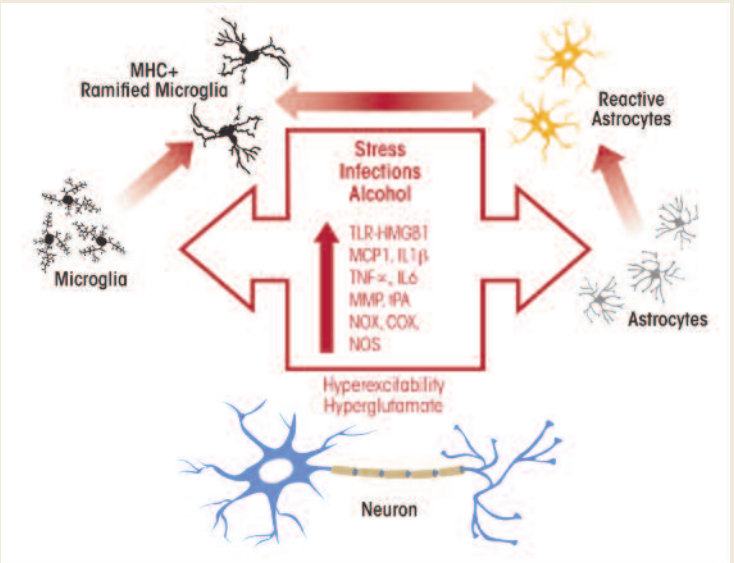

Figure 1 Activation of microglia and astrocytes by alcohol in the brain. Microglia and astrocytes undergo multiple stages of activation that include characteristic changes in morphology. Resting microglia become ramified microglia with that express molecules called major histocompatibility complex (MHC) on their surface. Similarly, astrocytes begin to show markers of reactive astrocytes. Alcohol-induced glial activation is associated with increased expression of innate immune genes, including increased expression of the chemokine monocyte chemoattractant protein-1 (MCP1); the cytokines tumor necrosis factor-α (TNFα), interleukin-1 β (IL-1β) , and interleukin-6 (IL-6); the proteases matrix metalloproteinase (MMP) and tissue plasminogen activator (TPA); and the oxidases nicotinamide adenine dinucleotide phosphate oxidase (NOX), cyclooxygenase (COX), and nitric oxide synthetase (NOS). The alcohol-induced activation of glial innate immune genes increases neuronal hyperexcitability (Crews et al. 2011).

[2] Glutamate transporters are proteins that shuttle glutamate released by nerve cells (i.e., neurons) into the space between cells back into the neuron; this is essential to terminate transmission of a nerve signal and thus ensure appropriate regulation of neuronal activity.

[3] Transcription factors are proteins that are necessary for a set of reactions called transcription, which is the first step of the process during which the genetic information encoded in the DNA is used as a template for the generation of functional proteins.

cortex (HEC) (Zou and Crews 2006). These and other studies also have indicated that ethanol increases transcription of NF-κB target genes, including the genes encoding the following:

- MCP-1;
- Certain proinflammatory cytokines, such as TNFα, IL-1β, and IL-6;
- Certain proinflammatory oxidases, such as iNOS (Zou and Crews 2010), COX-2 (Knapp and Crews 1999), and NOX (Qin et al. 2008); and
- Certain proteases, such as TNF–converting enzyme (TACE) and tissue plasminogen activator (Zou and Crews 2010).

Not only ethanol but also chronic stress increases brain NF-κB activation (Koo et al. 2010; Madrigal et al. 2002), as well as the levels of cytokines, prostaglandin,[4] and COX-2 (Madrigal et al. 2003), all of which have proinflammatory effects. Although acute stress–induced responses, such as elevated glucocorticoid levels, are anti-inflammatory by blocking NF-κB production, chronic elevation of glucocorticoid levels during cycles of stress and/or AOD abuse reverses these anti-inflammatory effects and indeed results in proinflammatory NF-κB activation in the frontal cortex (Munhoz et al. 2010). Thus, activation of NF-κB is a common molecular mechanism through which stress and AODs can induce innate immune genes.

Addiction and Neuroimmune Signaling

Alcoholism is a progressive disease related to repeated episodes of alcohol abuse that reduce the brain's behavioral control and decision-making ability; at the same time, increasing habitual

[4] Prostaglandins are lipid compounds that are produced by almost all cells in the body and have a variety of important physiological effects, including the regulation of inflammatory reactions.

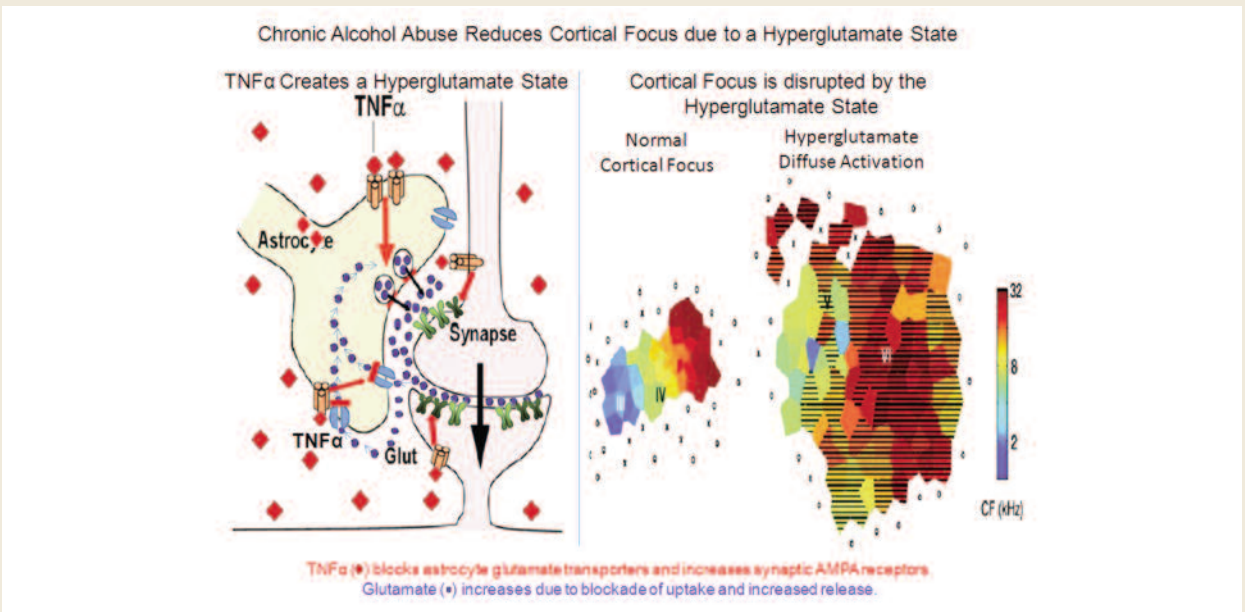

Figure 2 Mechanisms of alcohol-induced excessive glutamate activity in the cortex and loss of cortical focus. Ethanol-induced activation of microglia and astrocytes increases the levels of proinflammatory cytokines, including tumor necrosis factor-alpha (TNFα). (Left panel) TNFα creates a state characterized by excess activity of the neurotransmitter glutamate (i.e., a hyperglutamate state). Thus, TNFα reduces the levels of the primary glutamate transporters, GLT-1, in the astrocytes, in the cerebral cortex, and inhibits glutamate transport, possibly through induction of TNFα and other proinflammatory genes. As a result, glutamate levels outside the neurons, and particularly at the synapse, increase, resulting in a hyperglutamate state. In addition, TNFα increases the levels of certain molecules that interact with glutamate (i.e., AMPA receptors). All these processes causes excessive neuronal excitability. (Right panel) Hyperexcitability disrupts cortical focus. The left image shows the response of a normal adult auditory cortex to a series of tones with a frequence of 2-32 kHz colorized as blue to red. The response to a specific tone involves activation of a specific focal cortical region, which likely relates to the ability to distinguish specific tones of sounds. The right image shows the disrupted hyperglutamate-state–like response to sound that involves the entire auditory cortex without specific tonal areas of focus. The hyperglutatamate state increases cortical excitability, which in turn decreases function because it results in loss of focal activation and likely loss of tonal discrimination. In alcoholism, the hyperglutamate state most strongly affects the frontal cortex, which may disrupt decision making as well as attention and behavioral control mechanisms.

SOURCE: Image in right panel adapted from Chang and Merzenich (2003).

urges combined with increasing bad feelings (i.e., negative affect) promote continued drinking. Frontal cortical brain regions that designate attention and motivation, using information to predict the result of actions (Schoenbaum and Shaham 2008), play a role in addiction development. Frontal cortical dysfunction often is investigated using reversal-learning tasks. In reversal learning, the subject first learns to make one choice (e.g., responding to the black objects in a series of black and white objects) and then has to learn to reverse this choice (e.g., to respond to the white objects). Thus, the initially expected responses suddenly are considered wrong, requiring the subject to exhibit flexible behavior in response to outcomes that do not match those predicted by preceding cues (Stalnaker et al. 2009).

In behavioral studies, poor performance on such tasks is supposed to reflect the inability of drug-addicted individuals to learn new healthy behaviors and avoid the negative consequences of their drug consumption. Such learning and/or changes in behavior require signals from the frontal cortex to indicate the value of decisions. Studies found that binge drinking induces persistent deficits in reversal learning in rats (Obernier et al. 2002; Pascual et al. 2007) and in adult mice following a model of adolescent binge drinking (Coleman 2010). Other investigators similarly have demonstrated that cocaine use results in abnormally slow reversal learning, even though initial learning is normal (Calu et al. 2007; Schoenbaum et al. 2004). Specifically, human cocaine and alcohol addicts exhibit dysfunctional decision making in reversal-learning tasks that probe cognitive flexibility (Bechara et al. 2002). Lesions in the frontal cortex cause reversal-learning deficits comparable to those induced by chronic drug abuse (Schoenbaum et al. 2006). The persistence of addiction matches the persistent increases in innate immune gene activation (Qin et al. 2007, 2008) and loss of behavioral flexibility. Thus, it is thought that innate immune gene induction in the frontal cortex disrupts decision making consistent with addiction (Crews et al. 2011).

Addiction to alcohol, opiates, and stimulant drugs involves both changes in attention–decision making and increased temporal lobe anxiety–negative affect urgency. Addiction-induced negative affect and depression-like behaviors also are linked to neuroimmune signaling because neuroimmune signals can alter moods. For example, a compound called lipopolysaccharide (LPS) that can induce brain innate immune genes causes depression-like behavior that mimics components of addiction-like negative affect. LPS naturally binds with one of the TLRs (i.e., TLR4) and this interaction results in NF-κB activation, ultimately leading to the induction of innate immune genes. In humans, LPS infusions reduce reward responses and increase depressed mood (Eisenberger et al. 2010). Likewise, when patients with cancer or viral infections are treated with agents such as interferon and IL that influence innate immune genes, they may experience severe depression as a major adverse effect (Kelley and Dantzer 2011). Innate immune activators such as LPS, chemokines, and cytokines can mimic the amplification of depressed mood that occurs during repeated cycles of drug abuse or stress (Breese et al. 2008). All of these observations further support the link between neuroimmune signaling and mood as well as the role of neuroimmune signaling as a key component of addiction neurobiology. Of interest, chronic alcohol leads to withdrawal anxiety in normal mice

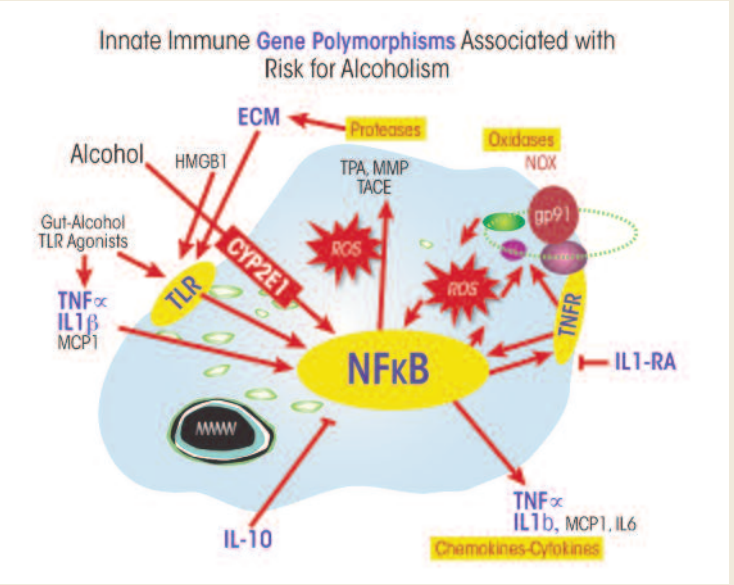

Figure 3 Innate immune gene polymorphisms associated with risk for alcoholism. The schematic shows a representative astrocyte or microglial cell. Genes associated with genetic risk for alcoholism are in light blue. Nuclear factor κ-lightchain–enhancer of activated B cells (NF-κB) is a key transcription factor involved in induction of innate immune genes that is sensitive to reactive oxygen species (ROS). These ROS are generated by the enzyme CYP2E1 during alcohol metabolism, and certain DNA sequences (i.e., polymorphisms) in the CYP2E1 gene are associated with alcoholism. CYP2E1 is highly expressed in monocyte-like cells, which are activated when CYP2E1 metabolizes alcohol. The ROS formed during this process activate proinflammatory NF-κB responses. Chronic ethanol treatment increases CYP2E1 expression in the brain, particularly in astrocytes.The resulting elevated ROS levels activate NF-κB–mediated transcription of innate immune genes, and this response may be amplified in the presence of certain NF-κB polymorphisms (i.e., NF-κB1). Certain variants of other genes also are associated with alcoholism, including polymorphisms of T NFα, interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1RA), and other components of the IL-1 gene complex, as well as of certain proteins in the space surrounding the cells (i.e., extracellular matrix proteins [ECM]).

tent with the hypothesis that innate immune activation drives negative affect and associated anxiety responses. Thus, the anxiety–depression negative affect that contributes to addiction occurs with increased brain neuroimmune signaling.

Neuroimmune signaling also regulates alcohol drinking behavior. Genetic comparisons among different strains of rats and mice found that addiction-like drinking behavior was associated with increased levels or activity of NF-κB, its regulatory proteins, and multiple innate immune genes (Mulligan et al. 2006). Furthermore, induction of innate immune genes resulted in increased ethanol consumption, whereas inactivation of such genes reduced drinking behavior (Blednov et al. 2005, 2011*b*). Thus, across genetically divergent strains of mice, innate immune responses to LPS corresponded to increases in ethanol consumption (Blednov et al. 2005, 2011*b*). In fact, even a single injection of LPS was able to produce a long-lasting increase in ethanol consumption (Blednov et al. 2011*a*) that corresponded to sustained increases in brain innate immune gene expression (Qin et al. 2007). These studies identified several innate immune molecules (e.g., β2-microglobulin, cathepsins, and CD14, a key innate immune signaling protein) as important for regulating drinking behavior. Thus, innate immune gene induction may underlie the progressive loss of behavioral flexibility, increasing negative affect, and increased alcohol drinking associated with repeat episodes of alcohol abuse and alcoholism.

Activity of Innate Immune Genes Is Increased in the Addicted Brain

Direct analyses of changes in the activity or levels of various proteins in the brains of alcoholics and other drug addicts also can provide insight into the neurobiology of addiction. Such studies found the following:

- Postmortem studies of the brains of human alcoholics indicate that the innate immune chemokine MCP-1 is increased severalfold in multiple brain regions (Breese et al. 2008). Consistent with this, chronic alcohol treatment of mice (Qin et al. 2008) or of cultured brain slices from the rat hippocampus (Zou and Crews 2010) also increases expression of MCP-1 and other innate immune genes.

- Proteins that serve as markers of microglial activation are increased across the alcoholic brain (He and Crews 2008).

- Consistent with alcoholism being related to neuroimmune signaling, postmortem studies of gene expression in the brains of human alcoholics found increased levels of a subunit of NF-κB; moreover, 479 genes targeted by NF-κB showed increased expression in the frontal cortex of alcoholics (Okvist et al. 2007).

- Postmortem analyses of alcoholic human brain gene expression found innate immune activation of cell adhesion and extracellular membrane components of innate immune gene signaling (Liu et al. 2006).

Thus, the findings of several studies of gene or protein expression are consistent with increased neuroimmune signaling in the brains of addicted individuals.

Polymorphisms of Innate Immune Genes and Genetic Risk of Addiction

Genetic factors account for approximately 50 percent of the risk of alcohol dependence (Schuckit 2009). Multiple genes linked to innate immune function also have been linked to the risk for alcoholism (see figure 3). DNA variations (i.e., polymorphisms) at specific locations on the chromosomes result in gene variants (i.e., alleles) that differ in their function or activity and thereby may increase or reduce the risk of alcoholism. For example, polymorphisms in the gene encoding an enzyme called CYP2E1, which is involved in ethanol metabolism, have been associated with the risk for alcoholism (Webb et al. 2010). In the body, CYP2E1 is highly expressed in monocyte-like cells; ethanol metabolism by CYP2E1 leads to the activation of these cells. Specifically, CYP2E1-mediated ethanol metabolism causes an increased production of highly reactive molecules called reactive oxygen species (ROS) within the monocytes that activate proinflammatory NF-κB responses (Cao et al. 2005) (see figure 3). In the brain, ethanol exposure leads to increased CYP2E1 expression, particularly in astrocytes (Montoliu et al. 1994, 1995), which likely contributes to astrocyte activation of NF-κB transcription during chronic alcohol exposure.

Human genetic association studies also have directly linked certain polymorphisms of the genes encoding NF-κB to alcohol dependence (Edenberg et al. 2008; Flatscher-Bader et al. 2005; Okvist et al. 2007). For example, polymorphisms in a precursor gene called *NF-kB1* that encodes one of the subunits of the transcription factor (i.e., the NF-κB p50 subunit) and which is important for activation of transcription have been associated with the risk for alcoholism (Edenberg et al. 2008). Likewise, alleles of the proinflammatory cytokine TNFα that result in increased TNFα expression have been linked to alcoholism and alcoholic liver disease (Pastor et al. 2000, 2005; Powell et al. 2000). Another genetic linkage exists between certain alleles of the anti-inflammatory, NF-κB–inhibiting cytokine IL-10 and alcoholism (Marcos et al. 2008). Additional genetic evidence regarding innate immune genes and the risk for alcoholism comes from polymorphisms of the gene encoding a molecule called the IL-1 receptor antagonist as well as from multiple other alleles of the IL-1 gene complex (Saiz et al. 2009).

In general, gene polymorphisms associated with increased risk of alcoholism tend to increase proinflammatory responses. For example, alcohol exposure may increase the expression of proinflammatory cytokines or individuals at risk of alcohol dependence may carry alleles associated with decreased anti-

inflammatory cytokine secretion. Thus, multiple innate immune gene polymorphisms are associated with genetic risk for alcoholism in humans, consistent with the assumption that increased brain innate immune gene expression contributes to the neurobiology of alcohol addiction.

Summary

The findings summarized in this article link innate immune gene induction to addiction and alcoholism. Monocytes, microglia, and astrocytes are sensitive to AODs and stress, with repeated AOD use causing progressive innate immune gene induction that parallels changes in decision making, mood, and alcohol consumption. Stress and AODs activate NF-κB transcription in the brain, which in turn enhances expression of proinflammatory NF-κB target genes. As a result, molecules related to the innate immune response, such as the chemokine MCP-1, the proinflammatory cytokines TNFα, IL-1β, and IL-6; the proinflammatory oxidases iNOS, COX, and NOX (Qin et al. 2008); and proinflammatory proteases are found following chronic ethanol treatment. Postmortem analyses of human alcoholic brain also have demonstrated increased expression of innate immune genes, which can disrupt cognition, mood, and drug consumption and is consistent with addition-like behavior. Finally, polymorphisms of genes involved in the innate immune responses influence the risk for alcoholism. These studies suggest that innate immune genes contribute to alcoholism and may be involved in the genetic risk for alcoholism. ■

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Alcohol Dependence and Genes Encoding α2 and γ1 $GABA_A$ Receptor Subunits

Insights from Humans and Mice

Cecilia M. Borghese, Ph.D., and R. Adron Harris, Ph.D.

One approach to identifying the causes of alcoholism, particularly without crossing ethical boundaries in human subjects, is to look at the person's genome (and particularly at the variations that naturally arise in the DNA) to identify those variations that seem to be found more commonly in people with the disease. Some of these analyses have focused on the genes that encode subunits of the receptor for the brain chemical (i.e., neurotransmitter) γ-aminobutyric acid (GABA). Different epidemiological genetic studies have provided evidence that variations in certain $GABA_A$ receptor ($GABA_A$-R) subunits, particularly subunits α2 and γ1, are correlated with alcohol dependence. Manipulations of these genes and their expression in mice and rats also are offering clues as to the role of specific $GABA_A$-Rs in the molecular mechanisms underlying alcoholism and suggest possibilities for new therapeutic approaches. KEY WORDS: Alcohol dependence; alcoholism; genetic factors; DNA; genetic theory of alcohol and other drug use (AODU); genetic vulnerability to AODU; genetic variants; γ-aminobutyric acid (GABA); $GABA_A$ receptor ($GABA_A$-R) subunits; GABRA2; GABRG1; single nucleotide polymorphisms (SNPs); ion channels; neurotransmitters; gene association studies; human studies; animal studies; mice; rats

Even though the consequences of alcohol dependence (AD) clearly are devastating and obvious to observers, the molecular mechanisms involved in the development of the disease are far from clear and understood. The search for these mechanisms is made even more difficult by the vast number of genes, proteins, and pathways in the human body that potentially could be involved, and by the obvious limitations of conducting research with human subjects without crossing ethical boundaries. Yet despite these complexities, various approaches already have allowed researchers to gather much knowledge in recent years, and the essential players in alcohol's mechanisms of action and in the development of AD already may have been identified. Thus, research has found that the primary targets of alcohol seem to be proteins prominently involved in neuronal communication, including:

- Ion channels in the neuronal membrane that are activated by signaling molecules (i.e., neurotransmitters) such as γ-aminobutyric acid (GABA) (i.e., $GABA_A$ receptors), glycine (i.e., glycine receptors), glutamate (i.e., *N*-methyl-D-aspartate receptors [NMDA-Rs]), acetylcholine (i.e., nicotinic receptors), and serotonin (i.e., $5\text{-}HT_3$ receptors);
- Ion channels regulated by changes in the electric potential across the neuronal membrane (i.e., voltage-gated channels), such as voltage-gated calcium channels; and
- Ion channels regulated by a type of regulatory molecules called G-proteins, such as G-protein–coupled inwardly rectifying potassium channels (GIRKs).

Alcohol's actions on these primary targets trigger the involvement of other systems that ultimately culminate in the development of dependence (Vengeliene et al. 2008).

Many techniques have yielded insight into alcohol's effects on the organism, but perhaps the most challenging field, given the logical ethical constrains, is the study of the neuronal structures and mechanisms that are affected by alcohol and/or which play a role in the development of AD in living humans. One way of circumventing these limitations is by studying how the natural variations (i.e., polymorphisms) between individuals in the genomic DNA relate to AD—that is, whether any specific variants are found more or less commonly than would be expected by chance in people with the disorder. This analysis can provide a glimpse of which genes or gene variants contribute to and shape the development of the disorder.

These natural differences in the genomic DNA between individuals arise from spontaneous mutations of single DNA building blocks (i.e., nucleotides) and are called single nucleotide polymorphisms (SNPs). (For more information on SNPs and their analysis, see the sidebar). In the past 10 years, different genetic association studies in alcohol-dependent subjects have identified several genes linked to this condition. Some examples of proteins that are encoded by genes in which the AD-linked SNPs are located include the following:

- The μ-opioid receptor (encoded by the OPRM1 gene) (Bart et al. 2005; Kim et al. 2004; Nishizawa et al. 2006; Ray and Hutchison 2004; Rommelspacher et al. 2001; Zhang et al. 2006);
- The κ-opioid receptor (OPRK1) (Edenberg et al. 2008; Xuei et al. 2006; Zhang et al. 2008);

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- Neuropeptide Y (NPY) (Ilveskoski et al. 2001; Lappalainen et al. 2002; Mottagui-Tabar et al. 2005);
- The muscarinic acetylcholine receptor M2 (CHRM2) (Dick et al. 2007; Luo et al. 2005; Wang et al. 2004); and
- The corticotropin-releasing hormone receptor 1 (CRHR1) (Chen et al. 2010).

Another group of genes related to alcohol dependence encode the $GABA_A$ receptors ($GABA_A$-Rs). This article will summarize what is known about the role of these receptors in the development of alcohol dependence.

$GABA_A$ Receptors

The $GABA_A$-Rs are proteins that span the membrane encasing the nerve cells (i.e., neurons) and which are composed of five subunits arranged around a central pore. There are several classes of subunits, including alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε), pi (π), theta (θ), and rho (ρ) subunits.

Single-Nucleotide Polymorphisms and Their Analysis

What Are Single-Nucleotide Polymorphisms?

Single-nucleotide polymorphisms (SNPs, pronounced "snips") are spontaneous mutations of single building blocks (i.e., nucleotides) in the genomic DNA. They can occur randomly, in any region of the DNA, including within those regions of the gene that actually encode parts of the resulting protein (i.e., coding sequences), within "silent" regions of a gene that ultimately do not encode parts of the resulting protein (i.e., non-coding regions), or in the regions between genes (i.e., intergenic regions). When a SNP occurs within a coding sequence, it may or may not change the amino acid sequence of the encoded protein. Each amino acid is represented by a three-nucleotide block of DNA (i.e., a codon). Because there are four different nucleotides (represented as A, C, G, and T), 64 possible codons exist; however, these encode only 20 amino acids. As a result, the genetic code is degenerate—that is, several codons may encode the same amino acid (e.g., both ACT and ACC encode threonine). A SNP in which both the original codon and the mutant codon produce the same protein sequence is called a synonymous polymorphism or silent mutation. If a different polypeptide sequence is produced, it is called a replacement polymorphism. This can result either in the introduction of a different amino acid, which is called a missense mutation, or in a premature stop of the protein, which is called a nonsense mutation (see the figure). Even if the SNP occurs in a noncoding region of the gene, it still may affect regulatory processes that could result, for instance, in altered protein levels.

When aligning DNA sequences from different individuals and comparing them at the same positions (i.e., loci) in the DNA, the occurrence of a SNP results in different "versions" of DNA called alleles. For example, in the figure, the two alleles for the SNP rs279868 are "A" and "G". Alleles frequently are transmitted from one generation to the next in a larger DNA block, usually from 5,000 to 100,000 nucleotides long. These blocks, which can contain numerous SNPs, are known as haplotypes. Thus, a haplotype specifies markers on one member of a pair of homologous chromosomes (i.e., either the chromosome inherited from the mother or the one inherited from the father).

Haplotypes are not always transmitted from one generation to the next, however, because of a process called recombination that randomly occurs during the formation of germ cells. As a result of recombination, new haplotypes should be formed based on the frequencies of the different alleles involved in the general population. Sometimes, however, certain combinations of alleles occur more or less frequently in a given population than would be expected from random formation of haplotypes. This nonrandom association of alleles at two or more loci is referred to as linkage disequilibrium. Identification of alleles that are in linkage disequilibrium can be useful for determining genes that are involved in conditions such as alcohol dependence.

How Can SNPs Be Analyzed?

Some genetic studies try to determine whether a certain allele (or haplotype) is present more or less frequently in people who suffer from a medical condition (e.g., alcoholism) than those without the disease; these are called genetic-association studies. One type of genetic-association studies are case–control studies, which include both individuals affected by the condition and disease-free control individuals from the same population. The frequency of alleles then is determined in both case and control subjects. Differences in the frequency of an allele between the two groups suggest that an association exists between the involved gene and the medical condition, with a certain allele conferring an increased or decreased risk for the condition. Identification of the precise nature of this association then requires additional studies.

units. The most commonly found $GABA_A$-Rs consist of two α, two β, and one γ or δ subunit. For some classes of subunits, several variants exist that are encoded by different genes, including six for the α subunit, three for the β subunit, and three for the γ subunit, allowing for numerous different subunit combinations. When GABA binds to the $GABA_A$-R, it activates the receptor and the central channel opens, allowing the entrance of negatively charged ions (i.e., anions), specifically Cl^-, to enter the neuron. This results in an increase in the difference in electrical charge between the inside and outside of the neuron (i.e., hyperpolarization), which in turn makes it more difficult for the neuron to transmit a nerve impulse, thus ultimately leading to inhibition of neuronal activity. Accordingly, GABA is considered an inhibitory neurotransmitter.

Considerable evidence points to the $GABA_A$-R as one of the main targets of alcohol (Kumar et al. 2009). The most abundant subunit combination in the brain, α1β2γ2, has been the most studied. Recently, the δ subunit–containing $GABA_A$-Rs also have been scrutinized in relationship to alcohol (Lobo and Harris 2008).

Single-Nucleotide Polymorphisms and Their Analysis *continued*

For example, the allele may alter the sequence, the splicing, or the levels of expression of the protein encoded or it may be in linkage disequilibrium with another allele that constitutes the genetic basis for the difference.

A caveat of these studies is that the frequencies of alleles/haplotypes can vary with ethnicity and geography; this is known as population stratification. One way to avoid this problem is to use family-based association designs. In this situation, unaffected family members (e.g., parents or siblings) are used as control subjects for the affected individuals. If an allele increases the risk of having the disease, then that allele would appear more frequently in the affected family members than in the unaffected members.

Other studies, such as twin and adoption studies, focus on the interaction between genes and environment. Twin studies compare the similarity of identical (i.e., monozygotic) and fraternal (i.e., dizygotic) twins. Identical twins generally are more similar than fraternal twins, because they are not only exposed to the same environment but also share a higher genetic similarity. In adoption studies, the adopted individuals are compared with control individuals (i.e., non-adopted individuals either from the adoptive family or the general population or adopted but unrelated children in the adoptive family). By comparing large numbers of twin pairs or adoptees and control subjects (e.g., with respect to the frequency of certain SNPs as well as the disease of interest), it is possible to better understand the role of genes and environment in the characteristics of a person.

Another approach to using SNPs to identify genes involved in a certain disease is to conduct genome-wide association studies (GWASs). With this strategy, a genetic association with the disease is investigated using many SNPs that cover the entire genome, instead of just a few genes as in the study approaches described above. This allows researchers to identify associations with genes that previously had not been expected to play a role in the disease under investigation. The downside of the GWASs is that they require a much larger number of subjects than do the other studies.

Segment of a single strand of DNA representing a fragment of the coding region from the *GABRA2* gene from two different people. There are two SNPs in this gene region—one in which both variants of the DNA encode the same amino acid (i.e., a silent mutation) and one in which the two variants of the DNA encode different amino acids (i.e., a missense mutation).

The genes encoding the $GABA_A$-R subunits are located in clusters on different chromosomes, including one cluster on chromosome 4 that carries genes called *GABRB1*, *GABRA4*, *GABRA2*, and *GABRG1*, which encode the β1, α4, α2, and γ1 subunits, respectively (see the figure). Previous human genetic studies have linked genetic polymorphisms in two regions of the *GABRA2* gene (i.e., in the middle and at the 3′ end of the gene) and in the region between the *GABRA2* and *GABRG1* genes (i.e., the *GABRA2* to *GABRG1* intergenic region) with AD (Agrawal et al. 2006; Covault et al. 2004; Edenberg et al. 2004; Enoch et al. 2006; Fehr et al. 2006; Lappalainen et al. 2005; Soyka et al. 2008). However, the evidence is not unequivocal, because other studies found no association between AD and the SNPs in this area (Covault et al. 2008; Drgon et al. 2006; Matthews et al. 2007). Even among the studies that did find a correlation, some inconsistencies existed. For instance, the first study of the association between *GABRA2* and AD came from the Collaborative Study on the Genetics of Alcoholism (COGA), a vast family-based association study (Edenberg et al. 2004). When Agrawal and colleagues (2006) extended the study of the COGA sample to include illicit drug dependence and comorbid dependence on alcohol and other drugs, the association was found only in subjects with AD and co-occurring drug dependence. On the other hand, Covault and colleagues (2004) found that the association was stronger when alcoholics with comorbid drug dependence were removed from the sample. Despite these inconsistencies, however, most of the clinical and genetic evidence points to *GABRA2* as a major genetic player in AD (see Enoch 2008).

All the SNPs that have been studied in the *GABRA2* and *GABRG1* genes to date are nonfunctional polymorphisms—that is, they do not alter the amino acid sequence of the encoded proteins. An alternative explanation for their role in the development of AD would be that the SNPs may alter the amount of protein that is produced. To address this possibility, researchers have analyzed the levels of an intermediate molecule called messenger RNA (mRNA) that is generated when the information encoded in the DNA is used for the production of a functional protein (i.e., during gene expression). An analysis of α2 mRNA levels in the prefrontal cortex of AD and control subjects found an association between α2 mRNA levels and the different variants (i.e., alleles) in the SNP rs279858,[1] although mRNA levels did not differ between control subjects and alcoholics (Haughey et al. 2008). Other recent data suggest that the apparent correlation between AD and *GABRA2* may result from a linkage disequilibrium with a not-yet-detected functional variant in the neighboring *GABRG1* gene. These findings on *GABRA2* and *GABRG1* and their association with AD are reviewed in the following sections, focusing on human studies and correlates in genetically modified rodents.

Analyses of the *GABRA2* and *GABRG1* Genes

Genetic-Association Studies

Several gene-association studies have examined the relationship of the *GABRA2* and *GABRG1* genes with AD, with varying results. Two studies—a large twin sample of the Australian population that also investigated the association with smoking and illicit drug use (Lind et al. 2008), and a small case–control study in an Italian sample (Onori et al. 2010)—reported no association between *GABRA2* and AD. Another case–control and family-association study (Sakai et al. 2010) sought to analyze the correlation between *GABRA2* genotype and substance abuse and behavioral problems in adolescents. The investigators only analyzed a single SNP in *GABRA2*, which was found not to be associated with conduct disorder or AD in adolescents.

Enoch and colleagues (2010) conducted a case–control study in African-American men with single and comorbid diagnoses of alcohol, cocaine, and heroin dependence, assessing the *GABRA2* genotype as well as childhood trauma. The exposure to childhood trauma predicted substance dependence.

[1] SNP rs279858 encodes a silent mutation (i.e., a mutation that does not result in an altered amino acid sequence) in the coding region of GABRA2.

[2] A haplotype is a set of closely linked genetic markers (e.g., SNPs) present on one chromosome that tends to be inherited together.

Figure Schematic representation of the cluster of $GABA_A$ receptor genes on chromosome 4. Arrows indicate gene position, size, and direction of transcription. The subunits and the names of the corresponding genes are α2 *(GABRA2)*, α4 *(GABRA4)*, β1 *(GABRB1)*, and γ1 *(GABRG1)*.

common in control subjects and seemed to confer resilience to addiction after exposure to severe childhood trauma. These findings suggest that in African-American men, childhood trauma, *GABRA2* SNPs, and their interaction determine (at least in part) the risk of or resilience to substance dependence. However, the data did not show a direct association between *GABRA2* and AD.

In a case–control adoption study of substance abuse (Philibert et al. 2009), the researchers determined the participants' genotypes for SNPs encompassing the *GABRA2* gene and analyzed them with respect to their history of alcohol, nicotine, and/or cannabis dependence. Both *GABRA2* genotype and haplotype were significantly related to vulnerability to all three types of substance dependence, particularly nicotine, and this association was more pronounced in female than in male subjects.

A small study using Japanese subjects (mostly social drinkers) examined the association between genetic variation in *GABRA2* (as assessed via seven SNPs) and subjective responses to alcohol as well as stimulant and sedative effects of alcohol (Roh et al. 2010). Three of these seven SNPs, all of which were located in the middle of the *GABRA2* gene, showed significant associations with subjective effects of alcohol. Specifically, individuals carrying one or two copies of the more common *GABRA2* allele (which is not associated with AD) showed greater subjective responses to alcohol than did individuals carrying two copies of the allele associated with AD. These results are, to some extent, in agreement with previous studies (Haughey et al. 2008; Pierucci-Lagha et al. 2005).

Another study (Kareken et al. 2010) examined the association between *GABRA2* SNPs and the brain's reward system. The participants, which included social drinkers, heavy drinkers, and alcohol-dependent individuals, first were assessed with respect to the brain's responses to alcohol cues (i.e., exposure to the odor of their preferred alcoholic beverage or a control odor) under both alcohol intoxication and control conditions using an imaging technique called functional MRI (fMRI). Then, the subjects were stratified according to their genotype at a SNP in *GABRA2* that previously had been shown to be associated with AD (Edenberg et al. 2004). All participants carried at least one copy of the high-risk allele of the SNP. Under both alcohol intoxication and control conditions, participants with two copies of this allele (i.e., homozygous subjects) exhibited a larger response to alcoholic odors than to control odors in one brain region (i.e., the medial frontal cortical areas), whereas participants with only one copy of this allele (i.e., heterozygous subjects) exhibited a larger response in another brain area (i.e., the ventral tegmental area). Thus, *GABRA2* variants seem to modify the activation of reward-related areas after exposure to alcohol-associated cues. Another study (Villafuerte et al. 2011) used fMRI to analyze the relationship between two *GABRA2* SNPs, the personality trait of impulsivity, and activation of a brain region called the insula cortex during anticipation of reward or loss in a family sample with high numbers of alcohol-dependent individuals. The investigators detected an association of all three variables, suggesting that *GABRA2* genotype influences insula responses and therefore impulsivity.

Another type of study called linkage disequilibrium analyzes whether certain alleles located close to each other on the same chromosome are inherited together more or less frequently than would be expected by chance alone.[3] Such studies in different populations have focused on *GABRA2* and either *GABRG1* (Ittiwut et al. 2008) or the intergenic region between the two genes (Philibert et al. 2009). The findings of these studies led to the conclusion that associations observed between *GABRA2* and the condition under investigation could be attributable to functional genetic variation at the *GABRG1* locus or that disease-related variants may exist at both loci.

Some new studies have focused on the *GABRG1* gene. Ray and Hutchinson (2009) examined associations between two SNPs of the *GABRG1* gene and alcohol use in hazardous drinkers. The data indicated that variation in one of the SNPs was associated with level of response to alcohol, drinking behavior, and alcohol problems.

Additional evidence of a significant *GABRG1* association with AD was found in a study involving Finnish Caucasian and Plains American Indians that examined both the *GABRA2* and *GABRG1* genes (Enoch et al. 2009). In both populations, there were significant haplotype and SNP associations of *GABRG1,* but not *GABRA2,* with AD. However, in the Finnish study population, the association of three less common haplotypes with AD was determined by *GABRA2*. Taken together, the findings of all of these studies suggest that independent contributions from both *GABRG1* and *GABRA2* likely contribute to the risk of AD.

Genome-Wide Association Studies

In contrast to the approaches used in the studies described above, genome-wide association studies (GWASs) investigate the genetic association with a disease using many SNPs that cover the entire genome instead of just a few genes. GWASs therefore also may discover associations with genes not previously suspected to be involved in the disease. One GWASs (Bierut et al. 2010) identified 15 SNPs that showed a significant association with AD. Moreover, when the investigators performed an independent evaluation for *GABRA2*, they found that five SNPs at that gene showed a modest association with AD.

Another GWASs was carried out in a case–control sample drawn from the families in the COGA, using individuals with AD (56 percent of whom also were dependent on illicit drugs) and individuals who used alcohol but were not dependent on alcohol or illicit drugs (Edenberg et al. 2010). The study identified no single SNP that met genome-wide criteria for significance; however, several clusters of SNPs provided mutual support for an association with the disease. An analysis of SNPs in genes encoding $GABA_A$-R subunits

[3] Even genes located close to each other on a chromosome may not always be inherited together, because of a process called genetic recombination that occurs with a certain probability during the generation of the germ cells.

in this sample found that a SNP in a gene called *GABRR2,* which encodes the $GABA_A$-R α2 subunit, was highly correlated with AD in this GWASs. This supported previous results, even though the level of significance was not high enough for genome-wide significance (Xuei et al. 2009). Likewise, SNPs in *GABRG1* were associated with AD, consistent with previous studies (Covault et al. 2008; Enoch et al. 2009). However, there was no evidence that the neighboring *GABRA2* gene was associated with AD. Finally, SNPs in other genes encoding $GABA_A$-R subunits (i.e., *GABRG3,* which encodes the γ3 subunit; *GABRA1,* which encodes the α1 subunit; and *GABRG2,* which encodes the γ2 subunit) also were associated with AD, again confirming findings of other investigators (Dick et al. 2004, 2006).

It is important to note that there are some inconsistencies in the findings of these genetic studies, which can be attributed to the inherent differences among the study types, the variability (i.e., heterogeneity) of the disease, and the genetic differences among the populations studied. In general, however, evidence continues to accumulate supporting an association between variations in genes encoding $GABA_A$-R subunits, particularly α2 and γ1 subunits, and AD.

Studies in Genetically Engineered Rodents

The studies in humans discussed above have been supplemented with studies of alcohol's effects on behavior in genetically engineered mice carrying mutations in GABA-related genes (Crabbe et al. 2006). For example, Boehm and colleagues (2004) found that mice in which the $GABA_A$-R α2 subunit had been deleted (i.e., α2 knockout mice) differed from control mice both in behavioral tests conducted without alcohol exposure (e.g., showed decreased spontaneous locomotion when tested for locomotor response to novelty) and in some behavioral responses to alcohol. Thus, the α2 knockout mice showed a shorter duration of alcohol-induced loss of righting reflex (LORR), which is a measure of alcohol's hypnotic effects. However, the sensitivity of the mice to acute ethanol withdrawal seemed unchanged, as did alcohol's anxiety-reducing (i.e., anxiolytic) effects. This lack of an effect of α2 deletion on alcohol's anxiolytic effects was unexpected, because mice genetically modified to possess a benzodiazepine-insensitive α2 subunit called α2(H101R) (i.e., knockin mice) no longer exhibited anxiolytic behavior when they were treated with the benzodiazepine diazepam (Low et al. 2000). Both alcohol and benzodiazepines are anxiolytic, and both increase $GABA_A$-R function. If both benzodiazepines and alcohol acted through α2-containing $GABA_A$-Rs to produce anxiolytic effects, deletion of the α2 subunit in the knockout mice should reduce alcohol's anxiolytic effects, and that did not happen. Furthermore, accurate assessment of alcohol's anxiolytic effects in the α2 knockout mice was complicated by the altered locomotor responses in these animals. Finally, female α2 knockout mice, but not males, preferred and consumed less alcohol than did the controls. However, interpretation of these findings is complicated by the female mice's greater aversion to bitter-tasting substances.

Another study was conducted in knockin mice carrying α1, α2, α3, or α5 $GABA_A$-R subunits that are insensitive to benzodiazepines through a mutation in a single amino acid (Tauber et al. 2003). The investigators administered diazepam and alcohol in combination to these mice and then determined the mice's LORR. All animals except for the α2 (H101R) mice showed similar sensitivity (i.e., increased LORR and reduced locomotor activity) to the combined drugs. Furthermore, the α2 (H101R) mice exhibited normal responses to alcohol alone (i.e., normal LORR and locomotor activity) and to a combination of low-dose alcohol and diazepam (i.e., normal locomotor activity). Thus, the benzodiazepine-induced increase in the alcohol-mediated hypnosis depends on the α2 $GABA_A$-R subunit.

Although null mutant mice that completely lack a certain $GABA_A$-R subunit provide important contributions to our knowledge of alcohol's targets, the deletion of a receptor in the brain, particularly an important one, is likely to trigger compensatory changes. For example, other receptor subunits could become more abundant and take on the functions of the missing subunit. An alternative approach is to design specific mutations that will render that receptor insensitive to alcohol but normal in every other aspect. One study compared the activities of $GABA_A$-Rs containing wild-type and mutated α2 subunits expressed in frog egg cells (i.e., *Xenopus* oocytes) (Blednov et al. 2011). With the normal $GABA_A$-Rs containing the wild-type subunit α2 (SL),[4] submaximal GABA responses were enhanced (i.e., potentiated) by alcohol. However, this potentiation was absent in the mutant α2 (HA)-containing $GABA_A$-Rs; there even was a small inhibition of the receptor's activity. In contrast, the mutation did not affect the receptor's sensitivity to GABA or the modulation by zinc, the benzodiazepine flunitrazepam, or the anesthetic etomidate (Werner et al. 2011).

On the basis of these findings, researchers developed and studied two corresponding mouse lines, the α2 SL/SL (i.e., wild-type) mice and the α2 HA/HA (i.e., knockin) mice. The responses to alcohol in these animals then were studied using a variety of tests (Blednov et al. 2011). The analyses found that some typical effects of alcohol (e.g., conditioned taste aversion and motor stimulation) were absent in the knockin mice. Moreover, the knockin animals showed changes in alcohol intake and preference in multiple tests as well as increased alcohol-induced hypnosis. In contrast, the knockin animals exhibited no changes in alcohol's anxiolytic and motor incoordination effects. These altered behavioral responses to alcohol in mutant (i.e., both knockout and knockin) mice may be related to altered subjective effects of alcohol in humans with different α2-associated SNPs (Kareken et al. 2010; Roh et al. 2010). In summary, the

[4] The wild-type α2 (SL) subunit carries the amino acid serine at position 270 and the amino acid leucine at position 277. In contrast, the mutant α2 (HA) subunit carried the amino acids histidine at position 270 and alanine at position 277.

study suggests that α2-containing $GABA_A$-Rs may be responsible for specific alcohol-induced effects. A subsequent study of the changes in mRNA levels induced by these mutations in the α2 subunit in the outer layer of the brain (i.e., the cerebral cortex) underlines the advantages of using knockin over knockout mice. Of almost 11,000 probes tested, the expression of only three genes was significantly modified in the knockin mice, and the behavioral responses to the sedative agents pentobarbital and flurazepam were unchanged (Harris et al. 2011). This confirms that the introduction of these mutations has minimal impact on the knockin animals compared with controls, minimizing the risk that effects unrelated to the behavior being investigated confound the results.

Another study in mice focused on the role of the $GABA_A$-R α2 subunit in changes in behavior produced by adaptation to chronic cocaine's effects (i.e., cocaine behavioral plasticity), such as locomotor sensitization, as well as in addiction (Dixon et al. 2010). In $GABA_A$-R α2 null mutant mice, cocaine did not induce a greater effect after repeated administration (i.e., did not produce behavioral sensitization) as it did in wild-type mice. Conversely, in mice carrying the benzodiazepine-insensitive $GABA_A$-R α2 (H101R) subunit, an agent called Ro 15–4513 that can increase the receptor responses in this mutant α2 subunit could stimulate locomotor activity if it was delivered into a brain region called the nucleus accumbens and induced behavioral sensitization to this effect after repeated administration. These results suggest that activation of α2-containing $GABA_A$-Rs in the nucleus accumbens is sufficient and necessary for behavioral sensitization. Furthermore, the investigators conducted a genetic case–control study in a diverse population (mainly Caucasian) that demonstrated an association of *GABRA2* with cocaine addiction in humans, emphasizing the relevance of α2-containing $GABA_A$-Rs in drug dependence.

Finally, researchers used an established animal model of human alcohol abuse, the selectively bred alcohol-preferring (P) rats, to look at the role of $GABA_A$-R subunits in alcohol's effects (Liu et al. 2011). The levels of $GABA_A$-R α1 subunits are elevated in a brain region called the ventral pallidum of these rats, and both α1 and α2 levels are increased in another region called the central nucleus of the amygdala (CeA). The study used molecules known as small-interfering RNAs (siRNA), which can interfere with gene expression, to specifically prevent production of α1 and α2 subunits. When siRNA targeted to α2 was infused into the CeA of P rats, both α2 expression and $GABA_A$-R density were reduced, and this was associated with inhibition of binge drinking. In contrast, siRNA targeted to α1 did not cause any of these changes when introduced in the CeA but did reduce α1 expression and binge drinking when administered into the ventral pallidum. These results highlight that not only the kind of $GABA_A$-R subunit but also the brain region in which it is located are relevant for alcohol consumption.

Implications of Genetic Findings for Therapeutic Approaches

Extending a previous study (Bauer et al. 2007), Das and colleagues (2010) analyzed the association between a SNP in the *GABRA2* gene and the efficacy of three psychotherapies for alcoholism (i.e., motivational enhancement therapy, cognitive–behavioral therapy, or 12-step facilitation) in preventing extreme drinking in AD patients. The study found that men with a high-risk *GABRA2* allele had a significantly higher probability of extreme drinking than did men without that allele. However, both men and women carrying at least one high-risk allele responded better to the therapy than did those who were homozygous for the low-risk allele. Among the female participants, the most effective therapy was cognitive–behavioral therapy, whereas among male subjects motivational enhancement therapy was most effective.

Tailoring pharmacotherapy to alcohol-dependent patients on the basis of genetic indicators also may be within reach. Two medications, naltrexone and acamprosate, currently are used for the treatment of alcoholism, but often with limited success. In an effort to identify potential associations between genotype and treatment outcome, Ooteman and colleagues (2009) determined SNPs in genes encoding different receptors involved in AD processes (i.e., opioid, dopamine, glutamate, and $GABA_A$ receptors) in alcohol-dependent individuals randomly assigned to acamprosate or naltrexone treatment. The investigators also quantified treatment effectiveness using tests administered the day before treatment initiation and on the last day the medication was administered. The tests included a cue exposure (i.e., participants were exposed to the sight and smell of their favorite alcoholic beverage while listening to a mood-induction script), followed by an assessment of self-reported cue-induced craving and physiological cue reactivity (i.e., heart rate). Significant association effects were found for several SNPs, suggesting that this may be the first step in matching patients to pharmacotherapy based on $GABA_A$-Rs and other genotypic markers.

Summary

Studies of genetically modified mice and rats have demonstrated that manipulation of α2 $GABA_A$-R subunits produces changes in alcohol-related phenotypes. The findings were more equivocal in human genetic studies, although strong evidence suggests that several $GABA_A$-R subunits, particularly α2 and γ1, have a role in AD in humans. Future studies hopefully will elucidate what exact mechanism creates these variations in the genetic code that affect AD, and how such variations can be used to provide a path to individualized therapy for patients with AD. ■

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Identifying Gene Networks Underlying the Neurobiology of Ethanol and Alcoholism

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For complex disorders such as alcoholism, identifying the genes linked to these diseases and their specific roles is difficult. Traditional genetic approaches, such as genetic association studies (including genome-wide association studies) and analyses of quantitative trait loci (QTLs) in both humans and laboratory animals already have helped identify some candidate genes. However, because of technical obstacles, such as the small impact of any individual gene, these approaches only have limited effectiveness in identifying specific genes that contribute to complex diseases. The emerging field of systems biology, which allows for analyses of entire gene networks, may help researchers better elucidate the genetic basis of alcoholism, both in humans and in animal models. Such networks can be identified using approaches such as high-throughput molecular profiling (e.g., through microarray-based gene expression analyses) or strategies referred to as genetical genomics, such as the mapping of expression QTLs (eQTLs). Characterization of gene networks can shed light on the biological pathways underlying complex traits and provide the functional context for identifying those genes that contribute to disease development. KEY WORDS: **Alcoholism; alcohol use disorders (AUDs); genetics; genetic basis of alcoholism; genetic technology; genetic association studies; quantitative trait loci (QTLs); genetic mapping; gene networks; genomes; genetical genomics; human studies; animal models**

The multiple genetic, environmental, and behavioral factors that play a role in the development of alcohol use disorders (AUDs) make it difficult to identify individual genes linked to these disorders. Nevertheless, some genetic risk factors (i.e., specific variants) associated with AUDs have been identified within many genes, some of which code for proteins involved in known biological pathways. Despite this progress, it has been exceedingly difficult to determine which genes may be the most relevant to developing therapeutic interventions for alcoholism. The major obstacles in treatment development are that gene–disease associations reveal very little about the underlying biology and that any implicated gene variant explains only a tiny proportion of an individual's overall risk for an AUD. Recent work focusing on the study of alcohol-related gene networks is helping to shed light on the molecular factors affecting alcoholism and other complex diseases. This article will provide an overview of approaches used to identify or construct gene networks and describe how systems biology approaches are helping to better understand complex traits such as behavioral responses to beverage alcohol (i.e., ethanol) and alcoholism.

Traditional Approaches to Dissecting Complex Traits

The predominant experimental strategy used by contemporary geneticists to identify the genetic factors involved in complex traits, such as behavioral responses to alcohol, essentially is an expansion of the gene mapping approach proposed by Botstein and colleagues (1980) over 30 years ago. For this approach, investigators scan their samples for genetic variations (i.e., polymorphisms) that segregate with the trait—that is, which are found in samples with the trait more often than would be expected by chance and therefore might contribute to the development of that trait. In recent human studies, this approach typically has been applied in genome-wide association studies (GWASs) of large, population-based samples that comprise both case subjects (i.e., individuals expressing the trait, or phenotype, under investigation) and unaffected control subjects. Hundreds of complex diseases and traits, including susceptibility to AUDs, have been analyzed using GWASs, resulting in the identification of several important links between genetic variants and these diseases (Bierut et al. 2010). Overall, however, the success of this approach has been mixed, and greater progress has been hindered by insufficient

sample sizes, stratified populations, the involvement of rare gene variants (i.e., alleles) that each only have a small effect size, and heterogenous phenotypic constructs (i.e., using different criteria to distinguish cases from controls).[1]

A similar forward-genetics approach that most often is used for studying animal models of complex traits is called quantitative trait locus (QTL) mapping. A quantitative trait is a phenotype that is determined by several genes, each of which has a variable contribution to the trait. The locations of the involved genes on the chromosomes are referred to as QTLs. QTL mapping studies typically are conducted using inbred strains of mice and their various derivatives. For example, the C57BL/6J (B6) and DBA2/J (D2) inbred mice frequently are used in alcohol research because they clearly differ in various responses to alcohol, including development of functional tolerance (Grieve and Littleton 1979), locomotor activation (Phillips et al. 1998), and sensitivity to withdrawal symptoms (Metten and Crabbe 1994). Because the environmental conditions in these experiments can be controlled, any differences observed between the mouse strains in these phenotypes most likely can be attributed to genetic differences. QTL mapping studies then seek to detect the polymorphisms underlying the complex traits of interest by scanning for alleles that co-vary with the traits.

Similar experiments also can be conducted with special derivatives of inbred strains known as recombinant inbred (RI) mice. These animals are derived by cross-breeding two or more distinct parental strains (which often diverge widely for the trait of interest), followed by inbreeding of the offspring for several generations (Bailey 1971). Given the correct breeding strategy, this method results in a panel of RI mouse strains that differ in the degree to which they exhibit a certain phenotype of interest. At the same time, each of the strains effectively is isogenic, meaning that for all genes, the genome carries two identical alleles (i.e., is homozygous). As a result, when two animals from the same RI mouse strain are bred, their offspring will have the exact same genetic makeup (i.e., genotype) as the parents. This makes it possible to directly integrate results generated from disparate experiments, in different laboratories, and at different times if they all use animals from the same RI mouse strain. This feature of RI mouse panels, and inbred animals in general, is particularly valuable for QTL mapping because the expense and time involved with genotyping or sequencing a strain only is incurred once.

The molecular and genetic resources outlined above have greatly increased the power and resolution of QTL mapping for various behaviors or other traits of interest. Yet despite these advances, the DNA regions identified as QTLs typically still are relatively large and may contain several genes; accordingly, few genes have been validated as contributing to quantitative traits (i.e., being quantitative trait genes [QTGs]). This difficulty is attributable largely to the lack of sufficient recombination events in existing mouse panels to reduce the size of DNA segments that typically are inherited together (i.e., haplotype block size) for fine mapping and to the generally small effect size for any single QTG.

Genomic Approaches to Disease Dissection

Because of the technical obstacles impeding their more effective use, both GWASs and QTL mapping studies to date have identified a deluge of disease-associated genetic loci but only few actual causal genes. Moreover, even the most successful studies have failed to place the disease-associated genes in any kind of biological context that would serve to explain the underlying functional biology. Without elucidating the complex interactions of the molecular phenotypes that stand between genetic variation and disease, it will be difficult or impossible to develop new and effective approaches to treating such diseases.

The emerging field of systems biology is tackling this immense challenge by studying networks of genes, proteins, metabolites, and other biomarkers that represent models of genuine biological pathways. Studying complex diseases in terms of gene networks rather than individual genes or genomic loci should aid in uncovering disease genes. With this approach, the effects of multiple genes in the network are combined, producing a stronger signal and reducing the number of statistical tests of association that must be performed.

These benefits effectively were demonstrated in two recent human association studies that modified the typical GWASs strategy by seeking associations only within groups of functionally related genes, rather than across the entire genome. The first of these studies (Ruano et al. 2010) discovered that cognitive ability, a complex phenotype with a large genetic component, was significantly linked to genes encoding molecules called G-proteins that consist of three different subunits (i.e., heterotrimeric G-proteins). The second study (Reimers et al. 2011) found that genes related to signaling pathways involving the neurotransmitters glutamate and γ-aminobutyric acid (GABA) signaling collectively contribute to alcohol dependence.

Network-based approaches to the dissection of complex diseases also can be applied to animal models, yielding experimental results that are more generalizable to humans because the pathways represented by these networks are more evolutionarily conserved than individual genes. This should encourage greater collaboration between researchers studying a common disease in different species. In fact, the biology underlying gene networks is so complex that any hope of deriving novel therapeutics may be entirely contingent on the extent to

[1] This is an issue faced by GWASs researchers when classifying samples as cases or controls. If cases are limited to only individuals who have been diagnosed with an AUD, it becomes difficult to enlist a sufficient number of participants. Moreover, many of the control subjects could very well be undiagnosed alcoholics or people who meet some but not all of the diagnostic criteria for an AUD. As a result, the control group could be polluted with near-cases, diluting any detectable group differences.

which scientists with diverse areas of expertise are willing to share and integrate datasets and make the process of interpretation a collaborative one.

Using High-Throughput Molecular Profiling to Define Disease

As the human and mouse genomes were being assembled using the cutting-edge, high-throughput DNA sequencers that made these endeavors possible, new technologies began to emerge that, for the first time, allowed near-comprehensive profiling of other cellular components. The term profiling refers to the measurement of different types of biological molecules, such as DNA to identify polymorphisms, messenger RNA (mRNA) to determine transcript abundance, proteins to identify certain chemical modifications that occur after the initial protein synthesis, and metabolites to evaluate biochemical processes in the cells. Platforms for high-throughput approaches for all these types of molecular profiling have become increasingly commonplace. Concurrently, methods for analyzing data produced by these technologies constantly are evolving, yielding results that are simultaneously more sensitive and more specific. As a result, researchers are better able to appreciate systems-level changes associated with disease.

Of these various high-throughput profiling techniques, microarray-based gene expression platforms have featured most prominently in biomedical research to date. Through an unbiased profiling of the transcriptome—that is, a measurement of all mRNA molecules produced within a cell or tissue sample—microarray expression studies allow researchers to identify patterns of gene expression associated with a disease. In some cases, such patterns can better define a complex phenotype by identifying disease subtypes. For example, microarray analysis of breast cancer tumors identified gene expression signatures that predict patient prognosis and therefore help physicians tailor treatment regimens (van't Veer et al. 2002). From a basic research perspective, microarray expression profiles can help tease apart the complex interactions that underlie the development of a disease by implicating a subset of genes whose regulation is altered with the disease. With this information, it may become feasible to reconstruct the underlying biological pathways and enhance understanding of disease etiology.

Genomic approaches have been applied to alcoholism directly by studying postmortem human brain tissue isolated from alcoholics and matched control subjects using gene expression microarrays. This has revealed novel information about changes in the brain's transcriptome that are associated with chronic ethanol consumption. One of the findings was a significant deregulation of genes encoding proteins that synthesize and maintain myelin, the substance that forms a sheath surrounding the long extensions (i.e., axons) of nerve cells and that is essential for effective nerve signal transmission (Lewohl et al. 2000; Mayfield et al. 2002). However, the nature of these studies makes it impossible to determine whether such gene expression deviations actually are risk factors that contribute to AUDs or simply represent molecular consequences of excessive alcohol consumption that are unrelated to the behaviors constituting alcoholism.

Animal models can assist greatly in this analysis by allowing for experiments that are far more detailed and informative but too invasive to ever be performed with humans. Although animal models could never replicate a phenotype as complex as alcoholism, they can mimic certain facets of the trait, which then can be associated with specific expression signatures using gene expression microarrays. For example, a genetic predisposition for alcoholism may entail a stronger-than-average preference for alcoholic beverages. This particular facet of alcoholism is captured by rodent models that selectively were bred to maximize a penchant for or aversion to ethanol, such as the aptly named high-alcohol preference (HAP) and low-alcohol preference (LAP) mice (Grahame et al. 1999). In order to identify genes that may alter the perceived desirability of ethanol, gene expression microarrays were used to compare the brain transcriptomes of HAP and LAP mice, as well as of several other inbred mouse strains that drastically differ in ethanol preference (Mulligan et al. 2006). This important study identified a diverse array of molecular pathways associated with differences in ethanol preference. Some of the genes that had the largest effect sizes were related to neuronal function and to the maintenance of the cells' normal internal conditions (i.e., cellular homeostasis).

Another important facet of a genetic predisposition to alcoholism is a comparatively blunted sensitivity to the effects of ethanol. Studies have shown that people who initially are less sensitive to acute ethanol exposure are more likely to have a family history of alcoholism and are at greater risk for developing an AUD (Schuckit 1984, 1994). As mentioned earlier, the B6 and D2 inbred mice frequently are used in genetic studies of ethanol sensitivity. For this reason, Kerns and colleagues (2005) used microarray expression studies to dissect the effect of acute ethanol exposure on the brain's transcriptome using the B6 and D2 inbred mouse strains. The investigators analyzed three brain regions involved in a brain system called the mesocorticolimbic reward pathway, which is involved in mediating the rewarding properties of alcohol and other drugs. For each region analyzed, the study identified a specific set of genes (i.e., a gene module) whose expression was altered in response to acute ethanol exposure. These gene modules contained greater-than-expected numbers of genes involved in several signaling pathways (i.e., retinoic acid signaling, neuropeptide expression, and glucocorticoid signaling). Moreover, similar to the microarray studies of postmortem human alcoholic brains (Lewohl et al. 2000; Mayfield et al. 2002), several genes involved in myelination robustly were altered by alcohol exposure, particularly in the prefrontal cortex (Kerns et al. 2005).

In examining the responses to acute or chronic alcohol exposure in rodent brains, these and numerous other genomic studies have enhanced the understanding of the "ethanol transcriptome" and provided a more comprehensive picture of the genes and molecular pathways that contribute to specific facets of AUDs than what is possible with studies of postmortem human brains (Daniels and Buck 2002; Mulligan et al. 2011; Rimondini et al. 2002; Saito et al. 2004; Treadwell and Singh 2004). Moreover, these studies effectively have demonstrated how gene expression microarrays can help close the information gap that exists between DNA variation and complex diseases. However, prioritizing the long lists of genes produced by comparative microarray studies conducted in either species has proven exceedingly difficult. As the costs associated with validating a given gene's role in driving a complex trait are considerable, an effective strategy for prioritizing candidate genes is crucial. Investigators therefore have used more systems-level approaches that combine genetic, genomic, and pharmacological methods to better delineate gene networks causally related to ethanol behaviors. Networks allow us to infer relationships between genes and determine which are most important.

The Gene Network As a Modern Genetic Map

The previous section mentioned several studies that used gene-expression microarrays to define lists of genes responding to ethanol or otherwise relevant to AUDs. Although these studies have provided important biological insights, the question of how such lists can be used to further advance understanding of a complex disease is not easily answered. Network-based approaches can greatly improve the interpretability of differential gene-expression results by providing information about the relationships between genes.

Networks are systems of interconnected components. For example, the World Wide Web is a global network of computers sharing documents connected by hyperlinks; road maps are renderings of city networks connected by highways; social networks are groups of people connected through friendships; cellular signaling pathways are groups of proteins connected through molecular interactions; et cetera. Placing such complex systems within a network framework makes it possible to formally analyze the relationships that constitute these systems. Gene networks typically are visualized as mathematical graphs—that is, a collection of vertices and edges, where genes are represented by nodes and the lines connecting the nodes indicate that some relationship exists between the genes.

Many published network analyses of gene groups use information about pre-existing biological relationships, which may be derived from sources such as literature co-citation analysis (i.e., genes mentioned together in a scientific abstract), protein–protein interaction databases, or gene ontology groupings. Some commercial tools are available for such studies, such as Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA). However, although these sources provide categories for interpreting the genomic data, they also force such interpretation into the mold of pre-existing information, thereby partially defeating the goal of genomic studies.

Genomic data collected with high-throughput molecular profiling presents the opportunity to derive novel gene–gene interactions. The maturity of gene expression microarrays relative to similar technologies designed to measure other molecular phenotypes on a genomic scale has meant that gene networks primarily are rendered as gene coexpression networks. In the context of gene coexpression networks, links between nodes typically indicate that the expression levels for two genes are strongly correlated with one another across whatever conditions an experiment entails (e.g., across tissues, time points, treatments, or individuals). Each link in a gene network essentially represents a testable hypothesis that can be validated through follow-up molecular experiments. Indeed, coexpression networks have been used to identify protein interactions that are novel (Scott et al. 2005) and conserved across species (Stuart et al. 2003).

Various novel and innovative methods exist for generating gene coexpression networks. Although a comprehensive review of these methods is beyond the scope of this article, a few select methods are described in more detail in the sidebar. In their simplest form, however, gene coexpression networks can be constructed by calculating Pearson correlations between all gene pairs and applying a cutoff threshold to determine which genes should be connected. The simplicity of this approach makes it an appealing choice for conducting a first round of analyses.

Wolen and colleagues (in press) have attempted to better define the mesocorticolimbic reward pathway's transcriptional response to acute ethanol exposure by expanding the original B6/D2 study (Kerns et al. 2005) to include members of the BXD family of recombinant inbred mouse strains. The naturally occurring DNA polymorphisms that distinguish each BXD strain cause heritable changes in gene expression, making it possible to identify genetically coregulated transcripts across the BXD family. Microarray expression data from the prefrontal cortex of BXD family animals were used to look for evidence of coregulation among the 307 ethanol-responsive genes identified in the original B6/D2 study (see figure 1). The analysis identified several groups of intercorrelated gene modules, indicating this gene set is comprised of several gene networks (figure 1).

A variety of calculations can be used to gauge the relative importance of a particular gene to the network as a whole (Horvath and Dong 2008). The simplest measurement of node importance is determined by the degree of "connectivity"—that is, the number of

other genes the node is connected to in the network. However, a gene's "position" in the network also is an important consideration. For example, a gene that served as the sole connection between two otherwise independent gene networks would rank fairly low on a priority scale based on connectivity alone, despite being an important channel of intermodule communication. A measurement of "betweenness centrality" (Girvan and Newman, 2002) can highlight such a gene by determining the frequency with which a node is included in the shortest paths between all possible node combinations.

Figure 2 highlights six subnetworks taken from the larger coexpression network depicted in figure 1. The network comprising genes known as *Mbp*, *Mobp*, *Mal*, and *Plp1* is of particular interest, because these genes all play a role in the formation and stabilization of myelin. In addition, a parallel analysis was conducted using microarray data only from the prefrontal cortex of the same BXD strains after they had received an injection of 1.8 g/kg ethanol into their abdominal cavity. This analysis revealed that the myelin gene network persisted but underwent minor topographical modifications. Most notably, additional connections were detected between *Plp1* and two additional genes called *Mog* and *Lpar1*. The absence of *Mog* in the original network probably was an artifact of the method used to form these networks, and the gene likely should have been included. *Plp1* and *Lpar1*, in contrast, were effectively unrelated at baseline and only showed evidence of coregulation after ethanol treatment, suggesting this is a genuine molecular response to ethanol (see figure 2B).

The *Lpar1* gene encodes a receptor for lysophosphatidic acid (LPA), a signaling molecule containing phosphate and lipid components (i.e., a phospholipid). Regulation of *Lpar1* is critical for proper nerve-cell formation (i.e., neurogenesis), including in a brain region called the hippocampus in adults (Matas-Rico et al. 2008). In addition, *Lpar1* regulates the breakdown of the myelin sheath (i.e., demyelination) that occurs after nerve injury (Inoue

Constructing Gene Networks

Various methods exist for generating gene networks. As mentioned in the main text, the simplest method for constructing gene coexpression networks involves calculating Pearson correlations for all pair-wise genes and applying a hard threshold to determine which genes should be connected. The robustness of these networks, initially referred to as "relevance networks," can be assessed through an approach called permutation testing (Butte et al. 2000). A more rigorous method for constructing gene coexpression networks utilizes a graph theoretical approach to identify densely intercorrelated gene modules called paracliques (Baldwin et al. 2005). Paracliques represent gene–gene interaction networks with extensive, but not perfect, strong expression correlations between all genes in the network (http://grappa.eecs.utk.edu/grappa/root). Paracliques can contain members with missing links. Therefore, paracliques provide an attractive compromise by augmenting coexpression with genes whose correlational relationships to a network are strong, but permissibly imperfect with a proportion of the network. This proportion, called the proportional glom factor, is a user-defined parameter.

A potential limitation of both relevance networks and paracliques is that they rely on hard thresholds to classify the relationship between genes as either connected or unconnected. The dichotomy imposed by this approach may be artificially limiting these networks, causing biologically meaningful relationships to be overlooked (Carter et al. 2004). For example, the absence of the *Mog* gene from the myelin network described in the main article and depicted in figure 2A following ethanol treatment is symptomatic of this limitation. An approach called weighted gene coexpression network analysis (WGCNA) is an increasingly popular method that avoids these potential pitfalls by utilizing a "soft-thresholding" approach to generate networks that conform to a scale-free topology (Zhang and Horvath 2005). Scale-free networks follow the power distribution they are named for, comprising many nodes that have sparse connections and a few that are highly interconnected. In addition to providing an accurate model for metabolic networks (Jeong et al. 2000), neural networks of the roundworm *Caenorhabditis elegans* (Watts and Strogatz 1998), and the World Wide Web (Albert and Jeong 1999), the scale-free topology also typifies gene coexpression networks (van Noort et al. 2004). Some researchers recently have used WGCNA to define correlated gene modules associated with blood alcohol levels using the "drinking-in-the-dark" paradigm of excessive ethanol consumption in B6 mice (Mulligan et al. 2011). WGCNA also can be implemented as a freely available package (Langfelder and Horvath 2008) for the R Statistical Environment and provides an excellent set of tutorials (available at genetics.ucla.edu/labs/horvath/CoexpressionNetwork/Rpackages/WGCNA).

Lpar1 regulates the breakdown of the myelin sheath (i.e., demyelination) that occurs after nerve injury (Inoue et al. 2004). The fact that *Lpar1* is brought into this network by ethanol exposure suggests the intriguing possibility that this gene may play a role in the loss of white matter[2] commonly observed in long-term alcoholic patients (Kril and Halliday 1999). This example illustrates how studying ethanol-induced changes in gene-network topology can produce testable hypotheses relevant to the neurobiology of alcoholism. Obviously, alterations in the gene network occurring after acute ethanol exposure might not always be relevant to alterations in brain structure and function (i.e., neural plasticity) or toxic effects that occur with chronic exposure, such as in alcoholism. Therefore, findings regarding networks relevant to one ethanol behavioral phenotype should be considered "specific" to that phenotype unless other genetic, pharmacological, or behavioral data suggests links to other aspects of ethanol's actions in animal models or humans. More generally, this example demonstrates how systems-level methods, like gene coexpression analysis, can help greatly expand the information content of gene expression microarray studies by filling in information about the gene–gene relationships.

Bridging the Gap Between Genomics and Gene Mapping

Genetical Genomics

Another important early advancement toward a more systems-level approach to identifying disease-associated genes was the application of gene mapping methods to high-throughput molecular data in order to identify causal links between molecular phenotypes and genomic regions. Like classical physiological or behavioral phenotypes, genetic factors influencing high-throughput measures of transcript, protein, and metabolite abundance can be identified by QTL mapping. To date, such analyses mostly have been applied to gene-expression microarrays, mapping gene expression QTLs (eQTLs). This largely is related to technical constraints, because whole-proteome expression profiling currently cannot be done with the same degree of sensitivity, coverage, and throughput as mRNA profiling.

The strategy of performing genetic linkage analysis on genome-wide molecular profiles was formalized and termed "genetical genomics" by Jansen and Nap (2001). This proposal primarily focused on gene-expression microarrays and posited that mapping eQTLs would enable researchers to construct robust gene networks as well as link these networks to metabolic or other phenotypes. The investigators also suggested that eQTL mapping could greatly aid in the identification of candidate genes underlying classical QTLs for disease traits. The first study to carry out QTL analysis across genome-wide gene expression microarrays was conducted using an experimental cross between two strains of the yeast *Saccharomyces cerevisiae* (Brem et al. 2002). Subsequently, several investigations applied the approach to mammalian systems (Schadt et al. 2003; York et al. 2005), including brain gene expression (Chesler et al. 2003, 2005).

These early genetical genomics studies also characterized the two major classes of eQTLs, labeled *cis* and *trans* eQTLs, which differ with respect to the position of the eQTL relative to the gene whose expression is altered (figure 3).

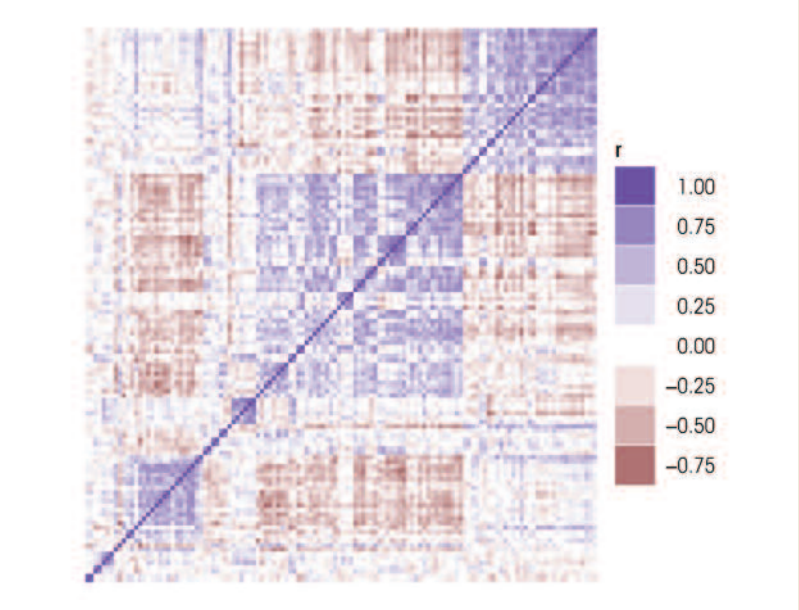

Figure 1 Correlation heatmap depicting patterns of co-expression among genes previously identified as being regulated by acute ethanol (Kerns et al. 2005). Each colored square represents the Pearson correlation (r) between a pair of genes, calculated using microarray expression data of prefrontal cortex tissue collected from B6, D2, and 27 BXD mouse strains. The blue and red colors indicate the strength and direction of the gene–gene correlation. Hierarchical clustering was applied to group genes based on the similarity of their expression profile across this dataset. In doing so, modules of co-expressed genes are revealed as cohesive blocks along the diagonal.

[2] The term "white matter" refers to brain structures made up primarily of nerve fibers that are enclosed by the myelin sheaths and therefore have a whitish appearance. Conversely, the term "gray matter" refers to brain structures composed mainly of the bodies of nerve cells, which have no myelin sheath, resulting in a grayish appearance.

A *cis* eQTL is located at the same site of the genome as the gene under study. In contrast, a *trans* eQTL can be located elsewhere in the genome, away from the gene whose expression is altered. A good example of how a *trans* eQTL could manifest involves transcription factors (TFs). These are proteins that bind with regulatory DNA regions that are located in front of a gene. Only when the TF binds to the corresponding DNA sequence can the first step in the process of gene expression—transcription—begin. The interaction between the TF and the DNA involves a certain part of the TF called the TF DNA–binding domain that allows the TF to recognize and bind with specific regulatory DNA sequences. Through this mechanism, certain TFs only may activate the transcription of specific sets of genes. Accordingly, a polymorphism at the DNA-binding domain of a certain TF can affect the TF's ability to recognize and bind its recognition sites, causing altered expression of all genes regulated by this TF. In other words, the abundance of all transcripts from those genes would co-vary with the TF polymorphism. Such a case might be recognized by a clustering of *trans* eQTLs at the site of the causal polymorphism, sometimes referred to as a regulatory hotspot. The identification of *trans* eQTL clusters can be a powerful approach for identifying key regulators underlying a complex trait of interest.

Figure 4 depicts the eQTL results for the same list of 307 ethanol-responsive genes identified in the B6/D2 study that earlier was used to construct coexpression networks. This analysis revealed that these coexpression networks share common eQTLs that drive this coordinated expression. Furthermore, the strongest eQTLs underlying many of these genes mapped to one end of chromosome 7, forming a *trans* eQTL cluster. These findings provide preliminary evidence that acute ethanol–responsive genes comprise a handful of gene coexpression networks in the prefrontal cortex and that a key regulator of these networks resides on chromosome 7. A more extensive analysis of this type has recently been completed (Wolen et al., in press).

The genes comprising *trans* eQTL clusters often have biological functions that have been conserved among species, suggesting that these hotspots may have a biological relevance. Accordingly, the search for *trans* eQTLs may allow researchers to identify biological functions associated with complex traits through defining quantitative trait gene networks (QTGNs). Mozhui and colleagues (2008) have, for example, dissected a *trans* eQTL cluster on distal mouse chromosome 1 and identified a candidate gene (*Fmn2*) that they

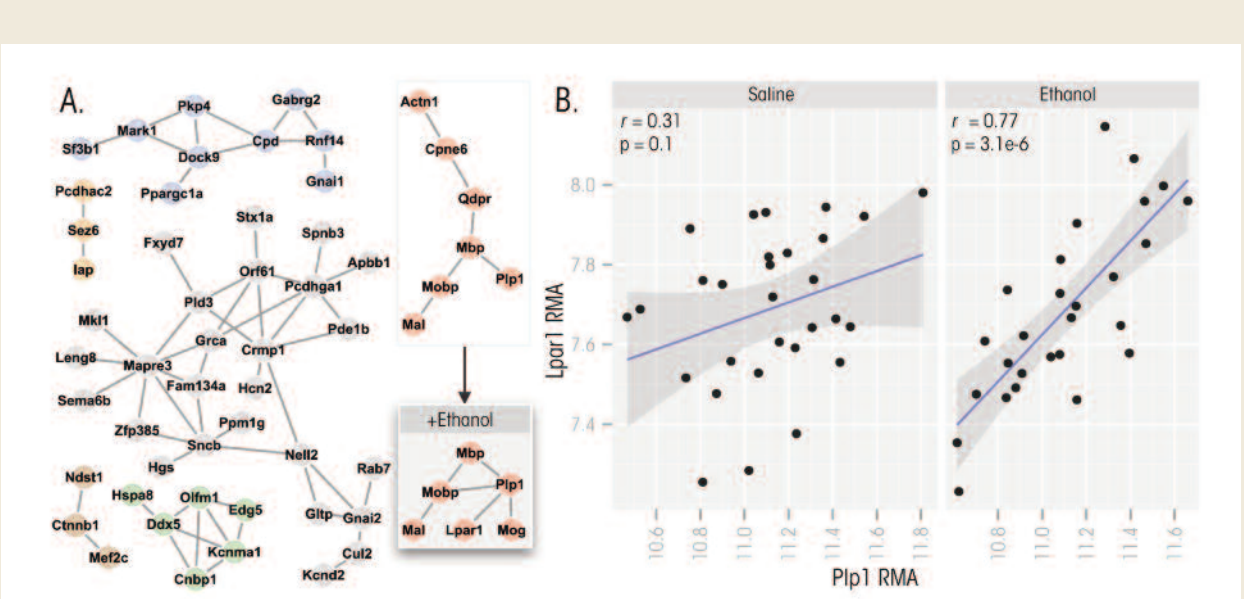

Figure 2 **A)** Gene networks that are regulated by acute alcohol exposure were identified in the same prefrontal cortex dataset used in Figure 1. Gene networks were generated by applying a hard threshold of 0.75 to the gene correlation matrix. The inset box contains a cognate of the myelin network (red) that was generated with expression data from the same strains following ethanol treatment. The ethanol-induced modifications of this network include the addition of a novel connection between *Plp1* and *Lpar1*. **B)** Scatterplots illustrating the correlation between *Plp1* and *Lpar1* at baseline and following ethanol treatment. The effective absence of any correlation between these genes at baseline suggests that this relationship is driven by ethanol exposure.

propose has a major influence on the expression of linked gene networks. Moreover, a diverse group of phenotypic QTLs seemed to be located in the same region, including several related to ethanol.

Genetical Genomics Studies to Identify Gene Variants Increasing Disease Risk

The integration of eQTL and classical QTL data enables identification of key markers of disease-causing variants. The effectiveness of this approach was demonstrated by a genetical genomics analysis of liver expression data from a population of mice placed on a high-fat diet (Schadt et al. 2003). The purpose of this diet was to model an obesity-like phenotype, which was measured using fat-pad mass (FPM). QTL mapping for FPM revealed a significant QTL on chromosome 2 that also harbored over 400 eQTLs. By scanning this region for *cis* eQTL-linked genes that also were strongly correlated with FPM, the researchers were able to identify two novel obesity candidate genes.

Saba and colleagues (2006) used a similar approach to identify candidate genes for alcohol preference and acute functional tolerance to alcohol. This large-scale study included rodent strains selectively bred for ethanol phenotypes (i.e., HAP and LAP mice) as well as a subset of the BXD family of recombinant inbred mice. Applying microarray expression profiles using mRNAs obtained from the entire brain, the investigators identified independent lists of genes whose expression differed between the HAP and LAP strains and between the BXD strains with high and low levels of acute functional tolerance. The genetic regulation of these gene lists then was mapped using BXD expression and genotypic data. High-priority candidate genes were highlighted by screening for differentially expressed genes with *cis* eQTLs that overlapped previously mapped behavioral QTLs for either alcohol preference (Belknap and Atkins 2001) or acute functional tolerance (Kirstein et al. 2002).

The rationale for prioritizing candidate QTGs on the basis of their having *cis* eQTLs located at the same sites as classical QTLs is based on the hypothesis that the variability of a complex phenotype is linked to a particular locus because the causal gene is being produced in variable quantities through a

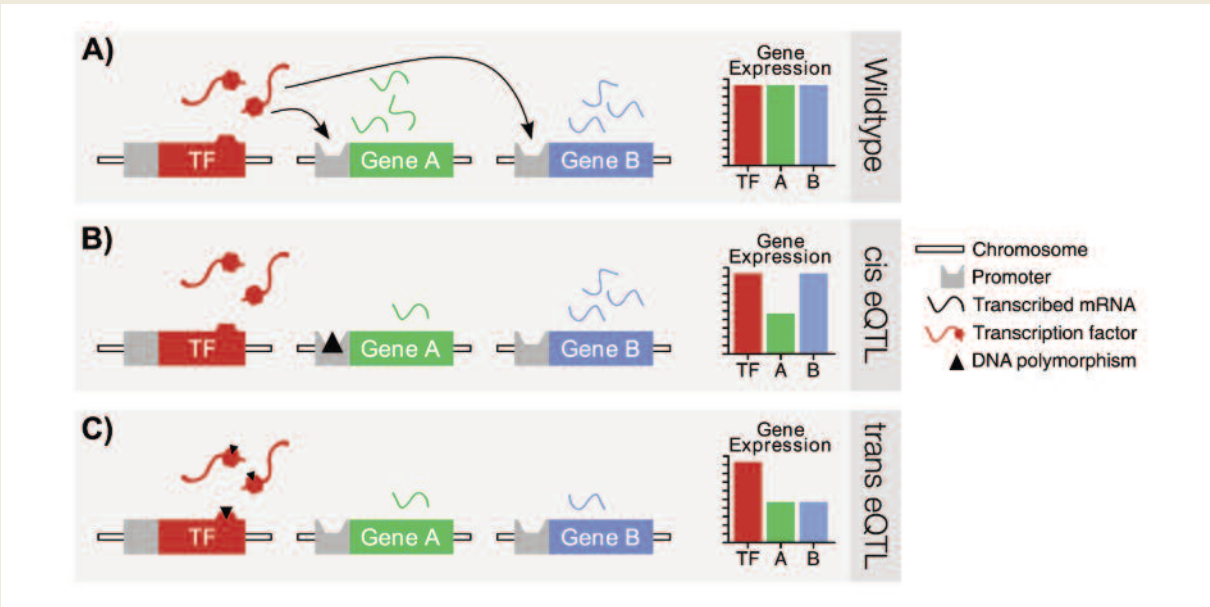

Figure 3 Illustration of the concept of *cis* and *trans* expression quantitative trait loci (eQTLs). **A)** The left-most gene (red) codes for a transcription factor (TF) protein that activates the transcription of genes A (green) and B (blue) by binding to their respective promoters (gray). In the wildtype, or "normal," scenario all genes are transcribed at their full potential, as indicated by the bar graph on the right. **B)** A variant (i.e., polymorphism) (triangle) in gene A's promoter region hinders TF binding, causing a reduction in the rate at which gene A is transcribed, while gene B is unaffected. Thus, gene A is being regulated by a *cis* eQTL because its level of expression is associated with a nearby polymorphism located on the same chromosome. **C)** A polymorphism in the TF gene's DNA binding region (hexagon)—the region of the TF protein that binds to gene promoters—hinders binding with all downstream promoters, regardless of whether the regulated gene is located near the TF gene, like gene A, or located on an entirely different chromosome, like gene B. In fact, all genes regulated by this TF would be linked to a *trans* eQTL at the site of this TF polymorphism.

cis-acting polymorphism. This hypothesis is somewhat of an oversimplification and leaves out several important caveats. Nevertheless, increasing evidence supports the importance of gene-expression variability in regulating complex traits. In fact, recent evidence indicates that SNPs associated with a variety of complex traits are more likely to contain *cis* eQTLs than normally would be expected (Nicolae et al. 2010). This indicates that the importance of expression variability in complex trait regulation is not limited to genetic model systems and that it may be possible for GWASs and QTL mapping studies to improve their track record by incorporating expression data.

Dissecting Complex Diseases Through Integrating Genomic Approaches

The discussion above has illustrated how traditional QTL mapping and GWASs approaches can benefit from systems-biological approaches by filling in critical information about the molecular phenotypes that stand between DNA variation and complex disease (figure 5). The incorporation of data from high-throughput molecular profiling technologies, such as gene expression microarrays, can better define a disease by identifying groups of genes that respond to or covary with disease-associated traits. Network analysis of disease-associated genes allows investigators to move beyond static gene lists, partially reconstruct the underlying molecular pathways, and prioritize genes based on their importance to the larger network. Applying QTL mapping to each gene's expression trait makes it possible to identify the genomic regions that regulate each gene's expression and uncover the existence of regulatory hotspots that exert enormous influence over a gene network. The series of studies discussed below has demonstrated how effective these methods are for dissecting complex traits, particularly when they are integrated.

Zhu and colleagues (2004) followed up the genetical genomics analysis of liver expression data from mice on a high-fat diet that was mentioned earlier (Schadt et al. 2003) by generating gene networks from the same microarray gene-expression dataset. This analysis included two distinct approaches to network construction: The first strategy formed networks on the basis of the covariation among gene-expression traits—that is, genes whose expression changed in the same manner were considered linked. In the second strategy, gene-network interactions were determined on the basis of the similarity of their eQTL profiles. Thus, the networks were constructed once without and once with the benefit of genotypic and eQTL data. Because links within gene networks represent causal relationships, analyses of these links can help researchers predict how a system will respond to the perturbation of a specific gene. Zhu and colleagues (2004) tested this hypothesis by measuring differential expression in response to a pharmacological substance that interfered with the function of a central gene in both predicted networks. They found that the eQTL profile network was a significantly better predictor of which genes would be affected by the pharmacological perturbation than the network constructed with expression data alone. Therefore, integrating both gene-expression and genotypic information into network construction greatly enhances the predictive value of gene networks.

The ultimate goal of systems-level analyses of complex diseases is to uncover

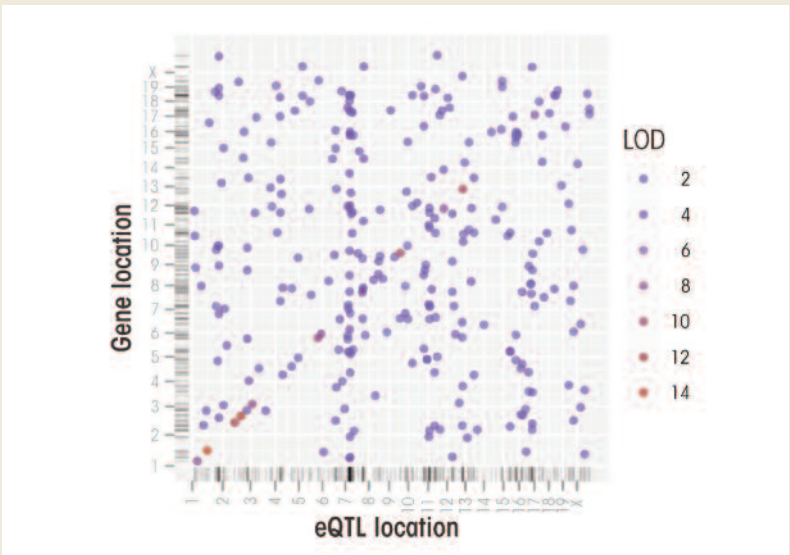

Figure 4 Genome–genome plot of peak expression quantitative trait loci (eQTLs) for the same dataset used in figure 1. Each point indicates the chromosomal position of a gene versus the position of its peak eQTL. Point color is used to communicate the strength of association between a gene and its eQTL, measured by logarithm of the odds (LOD) score. A LOD score is a ratio that measures that probability that a gene is linked to genetic markers, versus the probability that it is not. Thus, the higher a LOD score, the more likely a gene's expression level genuinely is regulated by an eQTL. Points plotted along the diagonal likely represent *cis* eQTLs, which also tend to have stronger LOD scores. Perpendicular tick-marks along both axes show the distribution of data points. Along the x-axis the dense clustering of tick-marks toward the proximal tip of chromosome 7 indicates the presence of a *trans* eQTL cluster, suggesting this region may harbor an important regulator of the gene co-expression modules seen in figure 1.

information necessary to establish a correlation between disease phenotype, mRNA abundance, and the underlying DNA polymorphism or causal gene network. Schadt and colleagues (2005) demonstrated this approach of integrating genotypic data, gene-expression data, and disease endophenotypes,[3] using the same liver expression dataset mentioned above, and a novel network construction technique termed likelihood-based causality model selection (LCMS). The investigators first identified all QTLs associated with a classical phenotype and then winnowed the list of potentially associated gene-expression traits on the basis of their correlation or eQTL overlap with the phenotype of interest. Candidate genes then were ranked by applying the LCMS technique, which uses the eQTL data to establish causal relationships between DNA loci and transcripts as well as between transcripts and phenotypes and finally identifies a model that best fits the data.

By ranking genes according to their performance in these models, the investigators identified several novel obesity candidate genes as well as uncovered additional support for the involvement of a gene called *Hsd11b1* that previously had been implicated in obesity risk (Rask et al. 2002). Because this gene seemed to be relevant to the phenotype they were investigating, the researchers then sought to reconstruct the gene network in which *Hsd11b1* participates by performing the LCMS procedure with *Hsd11b1* as the trait of interest. The resulting network was able to successfully predict genes that would be affected by inhibition of *Hsd11b1*. A similar approach has been used by other investigators to identify transcriptional networks associated with ethanol sensitivity behavior in the fruit fly *Drosophila melanogaster* (Morozova et al. 2011). This progression from phenotype to gene network to candidate gene and back to a gene network is a striking example of the promise that combining genetical genomics and gene-network analysis provides for understanding complex traits such as alcoholism.

As previously mentioned, such network-based techniques also have been applied to provide novel insight into the functional consequences associated with ethanol exposure in the mesocorticolimbic reward pathway. Preliminary results have identified several gene-coexpression networks that are robustly altered by ethanol in a tightly coordinated fashion (Wolen et al., in press). These studies used the BXD panel, so that the genetic regulation of ethanol-induced expression changes and behavioral responses also could be examined. Similar to results shown in figure 1, this analysis has revealed that ethanol-responsive gene networks are regulated by a small number of loci that largely are specific to a given network. At least one of these loci overlaps a previously mapped QTL for loss of righting reflex, a measure of acute ethanol sensitivity (Bennett et al. 2002; Markel et al. 1997). This work suggests that focusing on identifying gene networks both greatly reduces the complexity of whole-genome expression data and provides a wealth of hypotheses regarding both functional implications and regulatory mechanisms relevant to ethanol's action.

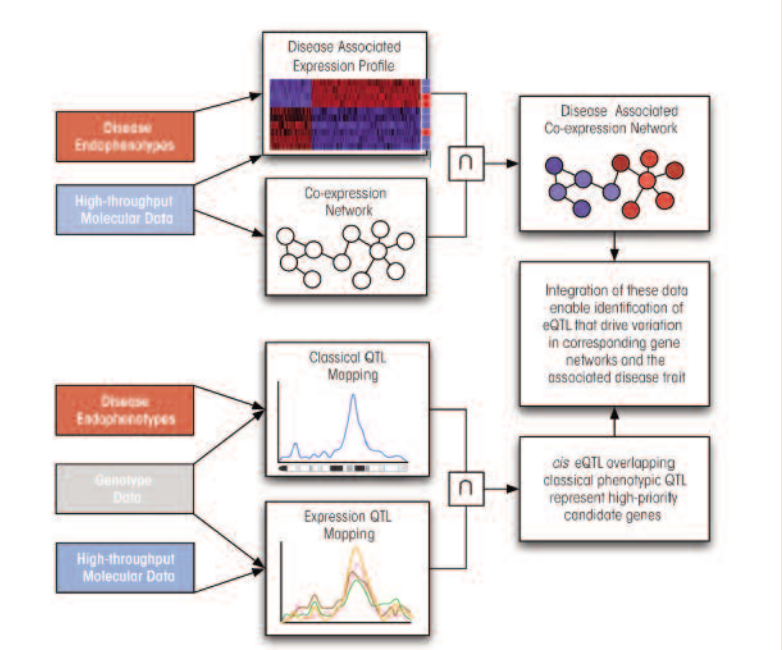

Figure 5 Diagram of how the genomic approaches discussed here can be integrated to identify gene networks and candidate genes for complex traits such as alcoholism. The information flow indicates how gene networks, expression quantitative trait locus (eQTL) and behavioral QTL analyses can be used together to identify candidate genes as potential targets for intervention. Note that resulting networks or candidate genes are entirely derived from experiments rather than relying on prior knowledge. In some cases, use of biomedical literature on gene–gene interactions can be used to augment such experimentally-derived networks.

[3] An endophenotype is a heritable trait or characteristic that is thought to be an intermediate phenotype between a genetic predisposition and a clinical disorder; for example, certain neurobiological characteristics have been noted in people with alcoholism and may be used as endophenotypes to identify people at risk for alcoholism. Endophenotypes are thought to be useful for gene identification under the assumption that they are simpler and closer to the genetic underpinnings of the disorder.

expression profiles associated with ethanol or alcoholism has provided modern neuroscience with a wealth of molecular information regarding ethanol's effects on the body. At the same time, alcohol researchers must make sense of a plethora of weak genetic signals and large lists of genes whose expression changes in response to alcohol. Newer approaches, such as exome[4] sequencing studies and certain approaches to analyzing gene expression (e.g., RNA-Seq analyses), promise added clarity but also may deliver even more confusing data. By combining behavioral, genetic, and genomic studies through genetical genomics and gene-network analysis designs, researchers may be able to construct gene networks rich in functional relations to ethanol behaviors. Additional refinements in ethanol-related gene-network structures and causal relation of such networks to aspects of ethanol-induced behaviors will provide a new generation of candidate genes for therapeutic intervention in alcoholism. ■

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[4] The exome is the part of the genome formed by the coding portions of genes (i.e., exons) that provide the genetic blueprint used in the synthesis of proteins and other functional gene products. It is the functionally most relevant part of the genome and, therefore, most likely to contribute to the phenotype of an organism.

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