ALCOHOL RESEARCH
Current Reviews

THE JOURNAL OF THE NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

Alcohol and Cannabinoids

Volume 42, Issue 1, 2022
Volume 41, Issue 1, 2021

National Institute on Alcohol Abuse and Alcoholism

TABLE OF CONTENTS

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2022;42(1):10 | https://doi.org/10.35946/arcr.v42.1.10 Published: 16 June 2022

Alcohol and Cannabinoids – From the Editors

Jane Metrik[1,2] and Sachin Patel[3]

[1]Center for Alcohol and Addiction Studies, Brown University School of Public Health, Providence, Rhode Island
[2]Providence Veterans Affairs Medical Center, Providence, Rhode Island
[3]Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Correspondence
Address correspondence concerning this article to Jane Metrik, Center for Alcohol and Addiction Studies, Brown University School of Public Health, Providence, RI, 02903. Email: Jane_Metrik@brown.edu

Acknowledgments
This work was supported by the National Institute on Alcohol Abuse and Alcoholism under grant number R01AA024091.

Disclosures
J.M. declares no competing financial or nonfinancial interests. S.P. is a scientific consultant for Psy Therapeutics and Jazz Pharmaceuticals.

Alcohol is frequently used in association with cannabis, with co-use now perceived as normative with expanding cannabis legalization. Cannabinoid products are increasingly used for a number of medical and recreational purposes, including to enhance alcohol-reinforcing properties or in some cases to substitute for alcohol. Rates of alcohol use disorder (AUD) are higher among cannabis users relative to nonusers, with approximately 60% of individuals with current cannabis use disorder also meeting criteria for current AUD.[1,2] Co-use is linked with heavy and problematic alcohol consumption, which in turn increases risk of alcohol-related diseases such as alcohol-associated liver disease. Co-use is also linked with a number of negative consequences, including behavioral risks,[3] risk for driving safety, psychiatric comorbidity, adverse health effects, and poor alcohol treatment outcomes.[4] However, the impact of cannabinoids on alcohol-related morbidity is not well understood, and findings on the impact of cannabis use on alcohol-related behaviors are equivocal. Cannabis serves both to complement drinking (i.e., increasing alcohol use), leading to more harmful consequences, and to substitute for alcohol effects (i.e., decreasing alcohol use and minimizing related risks).[5] Beyond simultaneous (i.e., same-session) or temporally independent (e.g., same-week) co-use patterns, the substantial variability in cannabinoid composition (i.e., tetrahydrocannabinol [THC]-to-cannabidiol ratio), formulations (e.g., smoked, edibles), and quantity of cannabis could influence the direction of effect on alcohol-related outcomes. Further, individual differences associated with age and neurodevelopment; substance use disorders;[6] motives for cannabis, alcohol, and simultaneous use;[7] and the impact of state-level cannabis and alcohol regulatory policies[8] could contribute to mixed findings on the risks and benefits of cannabinoids in relation to alcohol-related behaviors.

This research review series approaches cannabinoid–alcohol co-use through the lens of complex interactions between biological, psychological, and environmental factors. Basic science research reviewed in this topic series highlights the role of the endogenous cannabinoid or endocannabinoid (eCB) system in alcohol-related behaviors. The eCB system, which regulates cannabis reinforcement, is also involved in modulating alcohol reinforcement, motivation to consume alcohol, excessive alcohol consumption, AUD,[9-11] and alcohol-related diseases. Emerging preclinical literature implicates exogenous cannabinoid receptor agonists (e.g., THC) in increased alcohol consumption, with chronic exposure to alcohol implicated in disruptions in eCB signaling.[12,13] THC is the primary psychoactive constituent that interacts with the eCB system, producing intoxicating, rewarding, and reinforcing effects in a dose-dependent function. Although THC is the most commonly studied cannabinoid that defines cannabis potency, there are more than 100 other phytocannabinoids and more than 500 constituents in the cannabis plant that also may exert different effects on alcohol-related outcomes. For example, cannabidiol (CBD) is a nonpsychoactive, plant-based cannabinoid that has been implicated in the medicinal value of

cannabis due to its potential antioxidant, anti-inflammatory, and analgesic effects.

Cannabinoids may reduce harmful effects of AUD, in part, by conferring beneficial effects on the gastrointestinal and immune systems.[14-16] Endogenous cannabinoids, which are lipid molecules that exhibit cannabinoid-like properties, regulate various physiological functions in both the central nervous system and the peripheral organs, including the liver. Endocannabinoids and cannabinoid receptors in the liver modulate the progression of alcohol-related liver diseases via their effects on immune function, metabolic function, and inflammatory response.[17] Preclinical research on the efficacy of eCB degradation inhibitors indicates that these inhibitors show promise as an emerging therapeutic target for AUD and cannabis use disorder treatment.[18-20] Evidence from preclinical models also suggests CBD may have promise as a candidate pharmacotherapy for AUD.[21] CBD attenuates proximal alcohol-related behaviors (e.g., preference, stress-induced alcohol seeking) and reduces alcohol consumption[22,23] and alcohol-related physiological harms (e.g., liver steatosis and fibrosis, brain damage) in animal models.[24-26] Overall, there is growing recognition of the therapeutic potential of the eCB system in reducing negative affective states associated with AUD and with abstinence from alcohol in AUD patients.[27] Clinical studies on the acute and chronic impacts of specific cannabinoid and eCB targets on clinically relevant alcohol outcomes will help pave the way toward efficacious AUD pharmacotherapy and treatment of related medical conditions.

This translational research series strives to elucidate the cannabinoid–alcohol interactions by synthesizing findings across animal studies as well as human laboratory and epidemiological designs from community and clinical samples. From synapse to policy, the reviews in this series reflect the current state of the science on the reciprocal impact of alcohol and cannabinoids on an individual and the society at large. Several comprehensive reviews summarize findings from preclinical and human studies on the effects of alcohol exposure on the eCB system as a whole[28] and more specifically at the synaptic level in the brain.[29] In their review of the mechanisms of cannabinoid receptor signaling in hepatic pathogenesis, Yang, Choi, and Jeong summarize evidence in support of cannabinoid-based treatments for alcohol-associated liver disease.[30] Lees, Debenham, and Squeglia present a comprehensive overview of longitudinal neuropsychological and neuroimaging studies on the independent and combined effects of cannabis and alcohol use on the developing human brain.[31] Several articles review findings on the impact of cannabis use on alcohol consumption and consequences, and how this association may differ by cannabis formulation or by user characteristics,[32] with a specific focus on simultaneous alcohol and cannabis use, and contextual characteristics of co-use in young adults.[33] Finally, Pacula et al. provide a systematic review of published studies on the effect of liberalization of cannabis policies on alcohol use and co-use with cannabis in the United States and Canada.[34]

This topic series aligns with the research efforts discerning the shared impact of cannabinoids and alcohol on health undertaken by the Collaborative Research on Addiction at the National Institutes of Health (CRAN) partnership between the National Institute on Alcohol Abuse and Alcoholism, the National Institute on Drug Abuse (NIDA), and the National Cancer Institute. Elucidating effects of cannabis and alcohol co-use on health, policy, and economy is also a key research priority identified by the Cannabis Policy Research Workgroup of the NIDA National Advisory Council on Drug Abuse (NOT-DA-22-003). The empirical literature on cannabis and alcohol co-use has grown fourfold in the last decade alone, reflecting burgeoning interest in this topic. As summarized in the articles in this series, more research is needed to improve our understanding of the mechanisms underlying the functioning of eCBs in relation to alcohol in order to advance the development of eCB-based pharmacological treatments of AUD and related conditions. Clinical data examining the role of specific cannabinoids in alcohol-related human behavior also are critically needed to inform clinical guidelines for individuals engaged in AUD treatment and/or people who drink heavily and co-use cannabis. The authors lend crucial insights and make specific recommendations for future research endeavors on alcohol and cannabis interactions, taking into account between-person and within-person variability across time and contexts. All together, these findings will have important implications for the development of policy concerning alcohol in the context of the changing cannabis sociopolitical landscape.

References

1. Hayley AC, Stough C, Downey LA. DSM-5 cannabis use disorder, substance use and DSM-5 specific substance-use disorders: Evaluating comorbidity in a population-based sample. *Eur Neuropsychopharmacol.* 2017;27(8):732-743. https://doi.org/10.1016/j.euroneuro.2017.06.004.
2. Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality. *Results From the 2018 National Survey on Drug Use and Health: Detailed tables.* Rockville, MD: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. Published online 2019. https://www.samhsa.gov/data.
3. Jackson KM, Stevens AK, Sokolovsky AW, Hayes KL, White HR. Real-world simultaneous alcohol and cannabis use: An ecological study of situational motives and social and physical contexts. *Psychol Addict Behav.* 2021;35(6):698-711. https://doi.org/10.1037/adb0000765.
4. Subbaraman MS, Metrik J, Patterson D, Swift R. Cannabis use during treatment for alcohol use disorders predicts alcohol treatment outcomes. *Addiction.* 2017;112(4):685-694. https://doi.org/10.1111/add.13693.
5. Subbaraman MS. Substitution and complementarity of alcohol and cannabis: A review of the literature. *Subst Use Misuse.* 2016;51(11):1399-1414. https://doi.org/10.3109/10826084.2016.1170145.

6. Metrik J, Gunn RL, Jackson KM, Sokolovsky AW, Borsari B. Daily patterns of marijuana and alcohol co-use among individuals with alcohol and cannabis use disorders. *Alcohol Clin Exp Res*. 2018;42(6):1096-1104. https://doi.org/10.1111/acer.13639.
7. Patrick ME, Fairlie AM, Lee CM. Motives for simultaneous alcohol and marijuana use among young adults. *Addict Behav*. 2018;76:363-369. https://doi.org/10.1016/j.addbeh.2017.08.027.
8. Smart R, Pacula RL. Early evidence of the impact of cannabis legalization on cannabis use, cannabis use disorder, and the use of other substances: Findings from state policy evaluations. *Am J Drug Alcohol Abuse*. 2019;45(6):644-663. https://doi.org/10.1080/00952990.2019.1669626.
9. Colombo G, Serra S, Vacca G, Carai MAM, Gessa GL. Endocannabinoid system and alcohol addiction: Pharmacological studies. *Pharmacol Biochem Behav*. 2005;81(2):369-380. https://doi.org/10.1016/j.pbb.2005.01.022.
10. López-Moreno JA, Echeverry-Alzate V, Bühler K-M. The genetic basis of the endocannabinoid system and drug addiction in humans. *J Psychopharmacol*. 2012;26(1):133-143. https://doi.org/10.1177/0269881111416689.
11. Hungund BL, Basavarajappa BS. Role of endocannabinoids and cannabinoid CB1 receptors in alcohol-related behaviors. *Ann N Y Acad Sci*. 2004;1025(1):515-527. https://doi.org/10.1196/annals.1316.064.
12. Basavarajappa BS. Endocannabinoid system and alcohol abuse disorders. In: Bukiya A (ed.). *Recent Advances in Cannabinoid Physiology and Pathology. Advances in Experimental Medicine and Biology*. 2019;1162:89-127. https://doi.org/10.1007/978-3-030-21737-2_6.
13. Pava MJ, Woodward JJ. A review of the interactions between alcohol and the endocannabinoid system: Implications for alcohol dependence and future directions for research. *Alcohol*. 2012;46(3):185-204. https://doi.org/10.1016/j.alcohol.2012.01.002.
14. Karoly HC, Mueller RL, Bidwell LC, Hutchison KE. Cannabinoids and the microbiota–gut–brain axis: Emerging effects of cannabidiol and potential applications to alcohol use disorders. *Alcohol Clin Exp Res*. 2020;44(2):340-353. https://doi.org/10.1111/acer.14256.
15. Nair MP, Figueroa G, Casteleiro G, Muñoz K, Agudelo M. Alcohol versus cannabinoids: A review of their opposite neuro-immunomodulatory effects and future therapeutic potentials. *J Alcohol Drug Depend*. 2015;3(1):184.
16. Sharkey KA, Wiley JW. The role of the endocannabinoid system in the brain-gut axis. *Gastroenterology*. 2016;151(2), 252-266. https://doi.org/10.1053/j.gastro.2016.04.015.
17. Kunos G. Interactions between alcohol and the endocannabinoid system. *Alcohol Clin Exp Res*. 2020;44(4):790-805. https://doi.org/10.1111/acer.14306.
18. Zhou Y, Schwartz BI, Giza J, Gross SS, Lee FS, Kreek MJ. Blockade of alcohol escalation and "relapse" drinking by pharmacological FAAH inhibition in male and female C57BL/6J mice. *Psychopharmacology (Berl)*. 2017;234(19):2955-2970. https://doi.org/10.1007/s00213-017-4691-9.
19. Blednov YA, Cravatt BF, Boehm SL, Walker D, Harris RA. Role of endocannabinoids in alcohol consumption and intoxication: Studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology*. 2007;32(7):1570-1582. https://doi.org/10.1038/sj.npp.1301274.
20. Cippitelli A, Cannella N, Braconi S, et al. Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology (Berl)*. 2008;198(4):449-460. https://doi.org/10.1007/s00213-008-1104-0.
21. Turna J, Syan SK, Frey BN, et al. Cannabidiol as a novel candidate alcohol use disorder pharmacotherapy: A systematic review. *Alcohol Clin Exp Res*. 2019;43(4):550-563. https://doi.org/10.1111/acer.13964.
22. Gonzalez-Cuevas G, Martin-Fardon R, Kerr TM, et al. Unique treatment potential of cannabidiol for the prevention of relapse to drug use: Preclinical proof of principle. *Neuropsychopharmacology*. 2018;43(10):2036-2045. https://doi.org/10.1038/s41386-018-0050-8.
23. Viudez-Martínez A, García-Gutiérrez MS, Navarrón CM, et al. Cannabidiol reduces ethanol consumption, motivation and relapse in mice. *Addict Biol*. 2018;23(1):154-164. https://doi.org/10.1111/adb.12495.
24. Wang Y, Mukhopadhyay P, Cao Z, et al. Cannabidiol attenuates alcohol-induced liver steatosis, metabolic dysregulation, inflammation and neutrophil-mediated injury. *Sci Rep*. 2017;7(1):12064. https://doi.org/10.1038/s41598-017-10924-8.
25. Yang L, Rozenfeld R, Wu D, Devi LA, Zhang Z, Cederbaum A. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. *Free Radic Biol Med*. 2014;68:260-267. https://doi.org/10.1016/j.freeradbiomed.2013.12.026.
26. Hamelink C, Hampson A, Wink DA, Eiden LE, Eskay RL. Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. *J Pharmacol Exp Ther*. 2005;314(2):780-788. https://doi.org/10.1124/jpet.105.085779.
27. Bedse G, Centanni SW, Winder DG, Patel S. Endocannabinoid signaling in the central amygdala and bed nucleus of the stria terminalis: Implications for the pathophysiology and treatment of alcohol use disorder. *Alcohol Clin Exp Res*. 2019;43(10):2014-2027. https://doi.org/10.1111/acer.14159.
28. Serrano A, Natividad L. Alcohol-endocannabinoid interactions: Implications for addiction-related behavioral processes. *Alcohol Res*. 2022;42(1):09. https://doi.org/10.35946/arcr.v42.1.09.
29. Wolfe SA, Vozella V, Roberto M. The synaptic interactions of alcohol and the endogenous cannabinoid system. *Alcohol Res*. 2022;42(1):03. https://doi.org/10.35946/arcr.v42.1.03.
30. Yang K, Choi SE, Jeong W-I. Hepatic cannabinoid signaling in the regulation of alcohol-associated liver disease. *Alcohol Res*. 2021;41(1):12. https://doi.org/10.35946/arcr.v41.1.12.
31. Lees B, Debenham J, Squeglia, LM. Alcohol and cannabis use and the developing brain. *Alcohol Res*. 2021;41(1):11. https://doi.org/10.35946/arcr.v41.1.11.
32. Gunn RL, Aston ER, Metrik J. Patterns of cannabis and alcohol co-use: Substitution versus complementary effects. *Alcohol Res*. 2022;42(1):04. https://doi.org/10.35946/arcr.v42.1.04.
33. Lee CM, Calhoun BH, Abdallah DA, et al. Simultaneous alcohol and marijuana use among young adults: A scoping review of prevalence, patterns, psychosocial correlates, and consequences. *Alcohol Res*. 2022;42(1):08. https://doi.org/10.35946/arcr.v42.1.08.
34. Pacula RL, Smart R, Lira MC, Pessar SC, Blanchette JG, Naimi TS. Relationships of cannabis policy liberalization with alcohol use and co-use with cannabis: A narrative review. *Alcohol Res*. 2022;42(1):06. https://doi.org/10.35946/arcr.v42.1.06.

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2022;42(1):09 | https://doi.org/10.35946/arcr.v42.1.09 Published: 19 May 2022

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

Antonia Serrano[1,2] and Luis A. Natividad[3]

[1]Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain
[2]Unidad de Gestión Clínica de Salud Mental, Hospital Regional Universitario de Málaga, Málaga, Spain
[3]College of Pharmacy, Division of Pharmacology and Toxicology, University of Texas at Austin, Austin, Texas, USA

Correspondence
Address correspondence regarding this article to:

Antonia Serrano, Laboratorio Medicina Regenerativa, Hospital Regional Universitario de Málaga, Avda. Carlos Haya, 82, 29010 Málaga, Spain. Email: antonia.serrano@ibima.eu

Luis A. Natividad, Assistant Professor, College of Pharmacy, Division of Pharmacology and Toxicology, University of Texas at Austin, 107 W. Dean Keeton Street, C0875, Biomedical Engineering Building (BME) 6.114A, Austin, TX 78712, USA. Email: luis.natividad@austin.utexas.edu

Acknowledgments
This article was supported by Instituto de Salud Carlos III (ISCIII) and European Regional Development Funds–European Union (ERDF-EU), Ministerio de Economía y Competitividad (grants PI17/02026 and PI20/01399); Ministerio de Sanidad and Delegacion del Gobierno para el Plan Nacional sobre Drogas (grant PND2017/043); and a "Miguel Servet" research contract (CPII19/00031) funded by ISCIII and ERDF-EU.

This work was supported by National Institute on Alcohol Abuse and Alcoholism grant K99/R00AA025393.

The authors are ever grateful for the guiding hand of our dearly departed mentor and friend, Larry Parsons. His legacy in the endocannabinoid field continues to move us forward in our careers with much awe and inspiration for his achievements. Dr. Natividad would also like to dedicate this work in loving memory of his father, Pedro Natividad for his unconditional love and support.

Disclosures
The authors declare no competing financial or nonfinancial interests.

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,

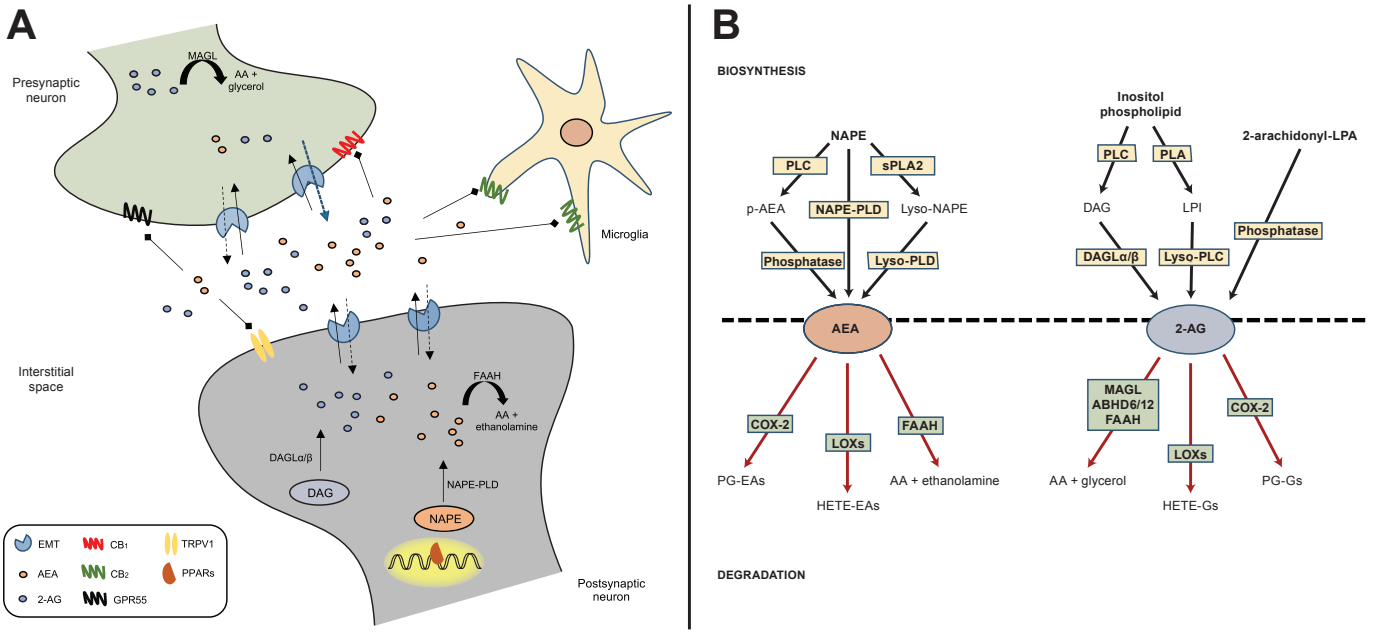

Figure 1. Endocannabinoid signaling and biosynthetic/degradation mechanisms. A: Schematic representation of the synaptic organization of the main components of the endocannabinoid system, including established routes of AEA and 2-AG metabolism. B: Metabolic pathways of synthesis and degradation of AEA 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.

References

1. Parsons LH, Hurd YL. Endocannabinoid signalling in reward and addiction. *Nat Rev Neurosci.* 2015;16(10):579-94. https://doi.org/10.1038/nrn4004.
2. Manzanares J, Cabañero D, Puente N, García-Gutiérrez MS, Grandes P, Maldonado R. Role of the endocannabinoid system in drug addiction. *Biochem Pharmacol.* 2018;157:108-121. https://doi.org/10.1016/j.bcp.2018.09.013.
3. Spanagel R. Cannabinoids and the endocannabinoid system in reward processing and addiction: From mechanisms to interventions. *Dialogues Clin Neurosci.* 2020;22(3):241-250. https://doi.org/10.31887/DCNS.2020.22.3/rspanagel.
4. Koob GF, Powell P, White A. Addiction as a coping response: Hyperkatifeia, deaths of despair, and COVID-19. *Am J Psychiatry.* 2020;177(11):1031-1037. https://doi.org/10.1176/appi.ajp.2020.20091375.
5. Basavarajappa BS. Endocannabinoid system and alcohol abuse disorders. *Adv Exp Med Biol.* 2019;1162:89-127. https://doi.org/10.1007/978-3-030-21737-2_6.
6. Basavarajappa BS, Joshi V, Shivakumar M, Subbanna S. Distinct functions of endogenous cannabinoid system in alcohol abuse disorders. *Br J Pharmacol.* 2019;176(17):3085-3109. https://doi.org/10.1111/bph.14780.
7. Herkenham M, Lynn AB, de Costa BR, Richfield EK. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* 1991;547(2):267-274. https://doi.org/10.1016/0006-8993(91)90970-7.
8. Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: A quantitative in vitro autoradiographic study. *J Neurosci.* 1991;11(2):563-583. https://doi.org/10.1523/jneurosci.11-02-00563.1991.
9. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993;365(6441):61-65. https://doi.org/10.1038/365061a0.
10. Van Sickle MD, Duncan M, Kingsley PJ, et al. Identification and functional characterization of brainstem cannabinoid CB_2 receptors. *Science.* 2005;310(5746):329-332. https://doi.org/10.1126/science.1115740.
11. Atwood BK, Mackie K. CB_2: A cannabinoid receptor with an identity crisis. *Br J Pharmacol.* 2010;160(3):467-479. https://doi.org/10.1111/j.1476-5381.2010.00729.x.
12. Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F. CB_2 receptors in the brain: Role in central immune function. *Br J Pharmacol.* 2008;153(2):240-251. https://doi.org/10.1038/sj.bjp.0707584.
13. Onaivi ES, Ishiguro H, Gong JP, et al. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann N Y Acad Sci.* 2006;1074:514-536. https://doi.org/10.1196/annals.1369.052.
14. Moreira FA, Jupp B, Belin D, Dalley JW. Endocannabinoids and striatal function: Implications for addiction-related behaviours. *Behav Pharmacol.* 2015;26(1-2):59-72. https://doi.org/10.1097/fbp.0000000000000109.
15. Di Marzo V, De Petrocellis L. Why do cannabinoid receptors have more than one endogenous ligand? *Philos Trans R Soc Lond B Biol Sci.* 2012;367(1607):3216-3228. https://doi.org/10.1098/rstb.2011.0382.
16. Pertwee RG, Howlett AC, Abood ME, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB_1 and CB_2. *Pharmacol Rev.* 2010;62(4):588-631. https://doi.org/10.1124/pr.110.003004.
17. O'Sullivan SE. Cannabinoids go nuclear: Evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol.* 2007;152(5):576-582. https://doi.org/10.1038/sj.bjp.0707423.
18. Overton HA, Babbs AJ, Doel SM, et al. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab.* 2006;3(3):167-175. https://doi.org/10.1016/j.cmet.2006.02.004.
19. Ryberg E, Larsson N, Sjögren S, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol.* 2007;152(7):1092-1101. https://doi.org/10.1038/sj.bjp.0707460.
20. Godlewski G, Offertáler L, Wagner JA, Kunos G. Receptors for acylethanolamides-GPR55 and GPR119. *Prostaglandins Other Lipid Mediat.* 2009;89(3-4):105-111. https://doi.org/10.1016/j.prostaglandins.2009.07.001.
21. Zygmunt PM, Petersson J, Andersson DA, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature.* 1999;400(6743):452-457. https://doi.org/10.1038/22761.
22. Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci.* 2003;4(11):873-884. https://doi.org/10.1038/nrn1247.
23. Liu J, Wang L, Harvey-White J, et al. Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology.* 2008;54(1):1-7. https://doi.org/10.1016/j.neuropharm.2007.05.020.
24. Bisogno T, Howell F, Williams G, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol.* 2003;163(3):463-468. https://doi.org/10.1083/jcb.200305129.
25. Sugiura T, Kondo S, Sukagawa A, et al. 2-Arachidonoylglycerol: A possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun.* 1995;215(1):89-97. https://doi.org/10.1006/bbrc.1995.2437.
26. Yamashita A, Kumazawa T, Koga H, Suzuki N, Oka S, Sugiura T. Generation of lysophosphatidylinositol by DDHD domain containing 1 (DDHD1): Possible involvement of phospholipase D/phosphatidic acid in the activation of DDHD1. *Biochim Biophys Acta.* 2010;1801(7):711-720. https://doi.org/10.1016/j.bbalip.2010.03.012.

27. Nakane S, Oka S, Arai S, et al. 2-Arachidonoyl-*sn*-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: Occurrence and rapid enzymatic conversion to 2-arachidonoyl-*sn*-glycerol, a cannabinoid receptor ligand, in rat brain. *Arch Biochem Biophys.* 2002;402(1):51-58. https://doi.org/10.1016/S0003-9861(02)00038-3.
28. McKinney MK, Cravatt BF. Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem.* 2005;74:411-432. https://doi.org/10.1146/annurev.biochem.74.082803.133450.
29. Dinh TP, Carpenter D, Leslie FM, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A.* 2002;99(16):10819-10824. https://doi.org/10.1073/pnas.152334899.
30. Gulyas AI, Cravatt BF, Bracey MH, et al. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci.* 2004;20(2):441-458. https://doi.org/10.1111/j.1460-9568.2004.03428.x.
31. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol.* 2007;14(12):1347-1356. https://doi.org/10.1016/j.chembiol.2007.11.006.
32. Marrs WR, Blankman JL, Horne EA, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci.* 2010;13(8):951-957. https://doi.org/10.1038/nn.2601.
33. Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S. Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett.* 1998;422(1):69-73. https://doi.org/10.1016/s0014-5793(97)01603-7.
34. Hermanson DJ, Hartley ND, Gamble-George J, et al. Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation. *Nat Neurosci.* 2013;16(9):1291-1298. https://doi.org/10.1038/nn.3480.
35. Rouzer CA, Marnett LJ. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: Cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem Rev.* 2011;111(10):5899-5921. https://doi.org/10.1021/cr2002799.
36. Solinas M, Justinova Z, Goldberg SR, Tanda G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J Neurochem.* 2006;98(2):408-419. https://doi.org/10.1111/j.1471-4159.2006.03880.x.
37. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev.* 2009;89(1):309-380. https://doi.org/10.1152/physrev.00019.2008.
38. Lovinger DM. Presynaptic modulation by endocannabinoids. *Handb Exp Pharmacol.* 2008(184):435-477. https://doi.org/10.1007/978-3-540-74805-2_14.
39. Marsicano G, Lutz B. Neuromodulatory functions of the endocannabinoid system. *J Endocrinol Invest.* 2006;29 (3 Suppl):27-46.
40. Chevaleyre V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci.* 2006;29:37-76. https://doi.org/10.1146/annurev.neuro.29.051605.112834.
41. Alger BE. Retrograde signaling in the regulation of synaptic transmission: Focus on endocannabinoids. *Prog Neurobiol.* 2002;68(4):247-286. https://doi.org/10.1016/s0301-0082(02)00080-1.
42. Sidhpura N, Parsons LH. Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. *Neuropharmacology.* 2011;61(7):1070-1087. https://doi.org/10.1016/j.neuropharm.2011.05.034.
43. Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron.* 2001;29(3):717-727. https://doi.org/10.1016/s0896-6273(01)00246-x.
44. Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron.* 2001;29(3):729-738. https://doi.org/10.1016/s0896-6273(01)00247-1.
45. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature.* 2001;410(6828):588-592. https://doi.org/10.1038/35069076.
46. Zlebnik NE, Cheer JF. Drug-induced alterations of endocannabinoid-mediated plasticity in brain reward regions. *J Neurosci.* 2016;36(40):10230-10238. https://doi.org/10.1523/JNEUROSCI.1712-16.2016.
47. Kalivas PW, O'Brien C. Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology.* 2008;33(1):166-180. https://doi.org/10.1038/sj.npp.1301564.
48. Kauer JA, Malenka RC. Synaptic plasticity and addiction. *Nat Rev Neurosci.* 2007;8(11):844-858. https://doi.org/10.1038/nrn2234.
49. Torregrossa MM, Corlett PR, Taylor JR. Aberrant learning and memory in addiction. *Neurobiol Learn Mem.* 2011;96(4):609-623. https://doi.org/10.1016/j.nlm.2011.02.014.
50. Adermark L, Lovinger DM. Frequency-dependent inversion of net striatal output by endocannabinoid-dependent plasticity at different synaptic inputs. *J Neurosci.* 2009;29(5):1375-1380. https://doi.org/10.1523/JNEUROSCI.3842-08.2009.
51. Clarke RB, Adermark L. Acute ethanol treatment prevents endocannabinoid-mediated long-lasting disinhibition of striatal output. *Neuropharmacology.* 2010;58(4-5):799-805. https://doi.org/10.1016/j.neuropharm.2009.12.006.
52. Adermark L, Jonsson S, Ericson M, Söderpalm B. Intermittent ethanol consumption depresses endocannabinoid-signaling in the dorsolateral striatum of rat. *Neuropharmacology.* 2011;61(7):1160-1165. https://doi.org/10.1016/j.neuropharm.2011.01.014.
53. Volkow ND, Fowler JS, Wang GJ, Swanson JM, Telang F. Dopamine in drug abuse and addiction: Results of imaging studies and treatment implications. *Arch Neurol.* 2007;64(11):1575-1579. https://doi.org/10.1001/archneur.64.11.1575.
54. DePoy L, Daut R, Brigman JL, et al. Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. *Proc Natl Acad Sci U S A.* 2013;110(36):14783-14788. https://doi.org/10.1073/pnas.1308198110.
55. Peñasco S, Rico-Barrio I, Puente N, et al. Intermittent ethanol exposure during adolescence impairs cannabinoid type 1 receptor-dependent long-term depression and recognition memory in adult mice. *Neuropsychopharmacology.* 2020;45(2):309-318. https://doi.org/10.1038/s41386-019-0530-5.
56. Hirvonen J, Goodwin RS, Li CT, et al. Reversible and regionally selective downregulation of brain cannabinoid CB_1 receptors in chronic daily cannabis smokers. *Mol Psychiatry.* 2012;17(6):642-649. https://doi.org/10.1038/mp.2011.82.
57. Ceccarini J, Kuepper R, Kemels D, van Os J, Henquet C, Van Laere K. [^{18}F]MK-9470 PET measurement of cannabinoid CB_1 receptor availability in chronic cannabis users. *Addict Biol.* 2015;20(2):357-367. https://doi.org/10.1111/adb.12116.
58. Sloan ME, Grant CW, Gowin JL, Ramchandani VA, Le Foll B. Endocannabinoid signaling in psychiatric disorders: A review of positron emission tomography studies. *Acta Pharmacol Sin.* 2019;40(3):342-350. https://doi.org/10.1038/s41401-018-0081-z.
59. Ceccarini J, Hompes T, Verhaeghen A, et al. Changes in cerebral CB_1 receptor availability after acute and chronic alcohol abuse and monitored abstinence. *J Neurosci.* 2014;34(8):2822-2831. https://doi.org/10.1523/JNEUROSCI.0849-13.2014.

60. Hirvonen J, Zanotti-Fregonara P, Umhau JC, et al. Reduced cannabinoid CB_1 receptor binding in alcohol dependence measured with positron emission tomography. *Mol Psychiatry.* 2013;18(8):916-921. https://doi.org/10.1038/mp.2012.100.
61. García-Baos A, Alegre-Zurano L, Cantacorps L, Martín-Sánchez A, Valverde O. Role of cannabinoids in alcohol-induced neuroinflammation. *Prog Neuropsychopharmacol Biol Psychiatry.* 2021;104:110054. https://doi.org/10.1016/j.pnpbp.2020.110054.
62. Basavarajappa BS, Hungund BL. Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor *N*-arachidonoylphosphatidylethanolamine in SK-N-SH cells. *J Neurochem.* 1999;72(2):522-528. https://doi.org/10.1046/j.1471-4159.1999.0720522.x.
63. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. *Biochim Biophys Acta.* 2000;1535(1):78-86. https://doi.org/10.1016/S0925-4439(00)00085-5.
64. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. *Eur J Pharmacol.* 2003;466(1-2):73-83. https://doi.org/10.1016/s0014-2999(03)01557-7.
65. Pava MJ, Woodward JJ. A review of the interactions between alcohol and the endocannabinoid system: Implications for alcohol dependence and future directions for research. *Alcohol.* 2012;46(3):185-204. https://doi.org/10.1016/j.alcohol.2012.01.002.
66. Vinod KY, Yalamanchili R, Xie S, Cooper TB, Hungund BL. Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid system. *Neurochem Int.* 2006;49(6):619-625. https://doi.org/10.1016/j.neuint.2006.05.002.
67. González S, Cascio MG, Fernández-Ruiz J, Fezza F, Di Marzo V, Ramos JA. Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. *Brain Res.* 2002;954(1):73-81. https://doi.org/10.1016/s0006-8993(02)03344-9.
68. González S, Valenti M, de Miguel R, et al. Changes in endocannabinoid contents in reward-related brain regions of alcohol-exposed rats, and their possible relevance to alcohol relapse. *Br J Pharmacol.* 2004;143(4):455-464. https://doi.org/10.1038/sj.bjp.0705963.
69. Malinen H, Lehtonen M, Hyytiä P. Modulation of brain endocannabinoid levels by voluntary alcohol consumption in alcohol-preferring AA rats. *Alcohol Clin Exp Res.* 2009;33(10):1711-1720. https://doi.org/10.1111/j.1530-0277.2009.01008.x.
70. Mitrirattanakul S, López-Valdés HE, Liang J, et al. Bidirectional alterations of hippocampal cannabinoid 1 receptors and their endogenous ligands in a rat model of alcohol withdrawal and dependence. *Alcohol Clin Exp Res.* 2007;31(5):855-867. https://doi.org/10.1111/j.1530-0277.2007.00366.x.
71. Rubio M, McHugh D, Fernández-Ruiz J, Bradshaw H, Walker JM. Short-term exposure to alcohol in rats affects brain levels of anandamide, other *N*-acylethanolamines and 2-arachidonoyl-glycerol. *Neurosci Lett.* 2007;421(3):270-274. https://doi.org/10.1016/j.neulet.2007.05.052.
72. Vinod KY, Maccioni P, Garcia-Gutierrez MS, et al. Innate difference in the endocannabinoid signaling and its modulation by alcohol consumption in alcohol-preferring sP rats. *Addict Biol.* 2012;17(1):62-75. https://doi.org/10.1111/j.1369-1600.2010.00299.x.
73. Buczynski MW, Parsons LH. Quantification of brain endocannabinoid levels: Methods, interpretations and pitfalls. *Br J Pharmacol.* 2010;160(3):423-442. https://doi.org/10.1111/j.1476-5381.2010.00787.x.
74. Henricks AM, Berger AL, Lugo JM, et al. Sex- and hormone-dependent alterations in alcohol withdrawal-induced anxiety and corticolimbic endocannabinoid signaling. *Neuropharmacology.* 2017;124:121-133. https://doi.org/10.1016/j.neuropharm.2017.05.023.
75. Ferrer B, Bermúdez-Silva FJ, Bilbao A, et al. Regulation of brain anandamide by acute administration of ethanol. *Biochem J.* 2007;404(1):97-104. https://doi.org/10.1042/BJ20061898.
76. Alvarez-Jaimes L, Stouffer DG, Parsons LH. Chronic ethanol treatment potentiates ethanol-induced increases in interstitial nucleus accumbens endocannabinoid levels in rats. *J Neurochem.* 2009;111(1):37-48. https://doi.org/10.1111/j.1471-4159.2009.06301.x.
77. Caillé S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH. Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. *J Neurosci.* 2007;27(14):3695-3702. https://doi.org/10.1523/JNEUROSCI.4403-06.2007.
78. Alvarez-Jaimes L, Polis I, Parsons LH. Regional influence of cannabinoid CB_1 receptors in the regulation of ethanol self-administration by Wistar rats. *Open Neuropsychopharmacol J.* 2009;2:77-85.
79. Serrano A, Pavon FJ, Buczynski MW, et al. Deficient endocannabinoid signaling in the central amygdala contributes to alcohol dependence-related anxiety-like behavior and excessive alcohol intake. *Neuropsychopharmacology.* 2018;43(9):1840-1850. https://doi.org/10.1038/s41386-018-0055-3.
80. Ceccarini J, Casteels C, Koole M, Bormans G, Van Laere K. Transient changes in the endocannabinoid system after acute and chronic ethanol exposure and abstinence in the rat: A combined PET and microdialysis study. *Eur J Nucl Med Mol Imaging.* 2013;40(10):1582-1594. https://doi.org/10.1007/s00259-013-2456-1.
81. Taraschi TF, Ellingson JS, Janes N, Rubin E. The role of anionic phospholipids in membrane adaptation to ethanol. *Alcohol Alcohol Suppl.* 1991;1:241-245.
82. Gustavsson L. Brain lipid changes after ethanol exposure. *Ups J Med Sci Suppl.* 1990;48:245-266.
83. Alling C, Rodriguez FD, Gustavsson L, Simonsson P. Continuous and intermittent exposure to ethanol: Effect on NG 108-15 cell membrane phospholipids. *Alcohol Alcohol Suppl.* 1991;1:227-231.
84. Colombo G, Serra S, Brunetti G, et al. Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. *Psychopharmacology (Berl).* 2002;159(2):181-187. https://doi.org/10.1007/s002130100887.
85. Vinod KY, Sanguino E, Yalamanchili R, Manzanares J, Hungund BL. Manipulation of fatty acid amide hydrolase functional activity alters sensitivity and dependence to ethanol. *J Neurochem.* 2008;104(1):233-243. https://doi.org/10.1111/j.1471-4159.2007.04956.x.
86. Wang L, Liu J, Harvey-White J, Zimmer A, Kunos G. Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. *Proc Natl Acad Sci U S A.* 2003;100(3):1393-1398. https://doi.org/10.1073/pnas.0336351100.
87. Kelai S, Hanoun N, Aufrere G, Beauge F, Hamon M, Lanfumey L. Cannabinoid-serotonin interactions in alcohol-preferring vs. alcohol-avoiding mice. *J Neurochem.* 2006;99(1):308-320. https://doi.org/10.1111/j.1471-4159.2006.04054.x.
88. Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB_1 receptor agonist. *Eur J Pharmacol.* 1999;370(3):233-240. https://doi.org/10.1016/s0014-2999(99)00170-3.
89. Malinen H, Hyytia P. Ethanol self-administration is regulated by CB1 receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. *Alcohol Clin Exp Res.* 2008;32(11):1976-1983. https://doi.org/10.1111/j.1530-0277.2008.00786.x.

90. Getachew B, Hauser SR, Dhaher R, et al. CB1 receptors regulate alcohol-seeking behavior and alcohol self-administration of alcohol-preferring (P) rats. *Pharmacol Biochem Behav.* 2011;97(4):669-675. https://doi.org/10.1016/j.pbb.2010.11.006.
91. Linsenbardt DN, Boehm SL 2nd. Agonism of the endocannabinoid system modulates binge-like alcohol intake in male C57BL/6J mice: Involvement of the posterior ventral tegmental area. *Neuroscience.* 2009;164(2):424-434. https://doi.org/10.1016/j.neuroscience.2009.08.007.
92. Alen F, Moreno-Sanz G, Isabel de Tena A, et al. Pharmacological activation of CB1 and D2 receptors in rats: Predominant role of CB1 in the increase of alcohol relapse. *Eur J Neurosci.* 2008;27(12):3292-3298. https://doi.org/10.1111/j.1460-9568.2008.06302.x.
93. Alén F, Santos Á, Moreno-Sanz G, et al. Cannabinoid-induced increase in relapse-like drinking is prevented by the blockade of the glycine-binding site of *N*-methyl-D-aspartate receptors. *Neuroscience.* 2009;158(2):465-473. https://doi.org/10.1016/j.neuroscience.2008.10.002.
94. Arnone M, Maruani J, Chaperon F, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB_1) receptors. *Psychopharmacology (Berl).* 1997;132(1):104-106. https://doi.org/10.1007/s002130050326.
95. Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci.* 1998;63(8):PL113-7. https://doi.org/10.1016/s0024-3205(98)00322-1.
96. Rodríguez de Fonseca F, Roberts AJ, Bilbao A, Koob GF, Navarro M. Cannabinoid receptor antagonist SR141716A decreases operant ethanol self-administration in rats exposed to ethanol-vapor chambers. *Zhongguo Yao Li Xue Bao.* 1999;20(12):1109-1114.
97. Economidou D, Mattioli L, Cifani C, et al. Effect of the cannabinoid CB_1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. *Psychopharmacology (Berl).* 2006;183(4):394-403. https://doi.org/10.1007/s00213-005-0199-9.
98. Freedland CS, Sharpe AL, Samson HH, Porrino LJ. Effects of SR141716A on ethanol and sucrose self-administration. *Alcohol Clin Exp Res.* 2001;25(2):277-282.
99. Lallemand F, Soubrié PH, De Witte PH. Effects of CB_1 cannabinoid receptor blockade on ethanol preference after chronic ethanol administration. *Alcohol Clin Exp Res.* 2001;25(9):1317-1323. https://doi.org/10.1111/j.1530-0277.2001.tb02353.x.
100. Cippitelli A, Bilbao A, Hansson AC, et al. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur J Neurosci.* 2005;21(8):2243-2251. https://doi.org/10.1111/j.1460-9568.2005.04056.x.
101. Serra S, Brunetti G, Pani M, et al. Blockade by the cannabinoid CB_1 receptor antagonist, SR 141716, of alcohol deprivation effect in alcohol-preferring rats. *Eur J Pharmacol.* 2002;443(1-3):95-97. https://doi.org/10.1016/s0014-2999(02)01594-7.
102. Pavon FJ, Bilbao A, Hernández-Folgado L, et al. Antiobesity effects of the novel in vivo neutral cannabinoid receptor antagonist 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1*H*-1,2,4-triazole – LH 21. *Neuropharmacology.* 2006;51(2):358-366. https://doi.org/10.1016/j.neuropharm.2006.03.029.
103. Gessa GL, Serra S, Vacca G, Carai MA, Colombo G. Suppressing effect of the cannabinoid CB_1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. *Alcohol Alcohol.* 2005;40(1):46-53. https://doi.org/10.1093/alcalc/agh114.
104. de Bruin NM, Lange JH, Kruse CG, et al. SLV330, a cannabinoid CB_1 receptor antagonist, attenuates ethanol and nicotine seeking and improves inhibitory response control in rats. *Behav Brain Res.* 2011;217(2):408-415. https://doi.org/10.1016/j.bbr.2010.11.013.
105. Dean RL, Eyerman D, Todtenkopf MS, Turncliff RZ, Bidlack JM, Deaver DR. Effects of oral loperamide on efficacy of naltrexone, baclofen and AM-251 in blocking ethanol self-administration in rats. *Pharmacol Biochem Behav.* 2012;100(3):530-537. https://doi.org/10.1016/j.pbb.2011.10.019.
106. Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J Neurochem.* 2003;84(4):698-704. https://doi.org/10.1046/j.1471-4159.2003.01576.x.
107. Naassila M, Pierrefiche O, Ledent C, Daoust M. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology.* 2004;46(2):243-253. https://doi.org/10.1016/j.neuropharm.2003.09.002.
108. Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND. Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB_1 receptors. *Behav Brain Res.* 2005;164(2):206-213. https://doi.org/10.1016/j.bbr.2005.06.021.
109. Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M. CB_1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacology.* 2005;30(2):339-349. https://doi.org/10.1038/sj.npp.1300568.
110. Henderson-Redmond AN, Guindon J, Morgan DJ. Roles for the endocannabinoid system in ethanol-motivated behavior. *Prog Neuropsychopharmacol Biol Psychiatry.* 2016;65:330-339. https://doi.org/10.1016/j.pnpbp.2015.06.011.
111. Pavon FJ, Polis I, Stouffer DG, et al. COX-2 inhibition antagonizes intra-accumbens 2-arachidonoylglycerol-mediated reduction in ethanol self-administration in rats. *Alcohol Clin Exp Res.* 2020;44(11):2158-2165. https://doi.org/10.1111/acer.14456.
112. Serrano A, Parsons LH. Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol Ther.* 2011;132(3):215-241. https://doi.org/10.1016/j.pharmthera.2011.06.005.
113. Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res.* 2011;50(2):193-211. https://doi.org/10.1016/j.plipres.2011.01.001.
114. Ishiguro H, Iwasaki S, Teasenfitz L, et al. Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. *Pharmacogenomics J.* 2007;7(6):380-385. https://doi.org/10.1038/sj.tpj.6500431.
115. Al Mansouri S, Ojha S, Al Maamari E, Al Ameri M, Nurulain SM, Bahi A. The cannabinoid receptor 2 agonist, beta-caryophyllene, reduced voluntary alcohol intake and attenuated ethanol-induced place preference and sensitivity in mice. *Pharmacol Biochem Behav.* 2014;124:260-268. https://doi.org/10.1016/j.pbb.2014.06.025.
116. Liu QR, Canseco-Alba A, Zhang HY, et al. Cannabinoid type 2 receptors in dopamine neurons inhibits psychomotor behaviors, alters anxiety, depression and alcohol preference. *Sci Rep.* 2017;7(1):17410. https://doi.org/10.1038/s41598-017-17796-y.
117. Navarrete F, García-Gutiérrez MS, Manzanares J. Pharmacological regulation of cannabinoid CB2 receptor modulates the reinforcing and motivational actions of ethanol. *Biochem Pharmacol.* 2018;157:227-234. https://doi.org/10.1016/j.bcp.2018.07.041.
118. Powers MS, Breit KR, Chester JA. Genetic versus pharmacological assessment of the role of cannabinoid type 2 receptors in alcohol reward-related behaviors. *Alcohol Clin Exp Res.* 2015;39(12):2438-2446. https://doi.org/10.1111/acer.12894.
119. Rivera P, Blanco E, Bindila L, et al. Pharmacological activation of CB_2 receptors counteracts the deleterious effect of ethanol on cell proliferation in the main neurogenic zones of the adult rat brain. *Front Cell Neurosci.* 2015;9:379. https://doi.org/10.3389/fncel.2015.00379.

120. Ortega-Álvaro A, Ternianov A, Aracil-Fernández A, Navarrete F, García-Gutiérrez MS, Manzanares J. Role of cannabinoid CB_2 receptor in the reinforcing actions of ethanol. *Addict Biol.* 2015;20(1):43-55. https://doi.org/10.1111/adb.12076.
121. Pradier B, Erxlebe E, Markert A, Rácz I. Interaction of cannabinoid receptor 2 and social environment modulates chronic alcohol consumption. *Behav Brain Res.* 2015;287:163-171. https://doi.org/10.1016/j.bbr.2015.03.051.
122. Cravatt BF, Demarest K, Patricelli MP, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A.* 2001;98(16):9371-9376. https://doi.org/10.1073/pnas.161191698.
123. Basavarajappa BS, Yalamanchili R, Cravatt BF, Cooper TB, Hungund BL. Increased ethanol consumption and preference and decreased ethanol sensitivity in female *FAAH* knockout mice. *Neuropharmacology.* 2006;50(7):834-844. https://doi.org/10.1016/j.neuropharm.2005.12.005.
124. Blednov YA, Cravatt BF, Boehm SL 2nd, Walker D, Harris RA. Role of endocannabinoids in alcohol consumption and intoxication: Studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology.* 2007;32(7):1570-1582. https://doi.org/10.1038/sj.npp.1301274.
125. Pavón FJ, Serrano A, Stouffer DG, et al. Ethanol-induced alterations in endocannabinoids and relevant neurotransmitters in the nucleus accumbens of fatty acid amide hydrolase knockout mice. *Addict Biol.* 2019;24(6):1204-1215. https://doi.org/10.1111/adb.12695.
126. Hansson AC, Bermúdez-Silva FJ, Malinen H, et al. Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology.* 2007;32(1):117-126. https://doi.org/10.1038/sj.npp.1301034.
127. Cippitelli A, Cannella N, Braconi S, et al. Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology (Berl).* 2008;198(4):449-460. https://doi.org/10.1007/s00213-008-1104-0.
128. Stopponi S, Fotio Y, Domi A, et al. Inhibition of fatty acid amide hydrolase in the central amygdala alleviates co-morbid expression of innate anxiety and excessive alcohol intake. *Addict Biol.* 2018;23(6):1223-1232. https://doi.org/10.1111/adb.12573.
129. Natividad LA, Buczynski MW, Herman MA, et al. Constitutive increases in amygdalar corticotropin-releasing factor and fatty acid amide hydrolase drive an anxious phenotype. *Biol Psychiatry.* 2017;82(7):500-510. https://doi.org/10.1016/j.biopsych.2017.01.005.
130. Zhou Y, Schwartz BI, Giza J, Gross SS, Lee FS, Kreek MJ. Blockade of alcohol escalation and "relapse" drinking by pharmacological FAAH inhibition in male and female C_{57}BL/6J mice. *Psychopharmacology (Berl).* 2017;234(19):2955-2970. https://doi.org/10.1007/s00213-017-4691-9.
131. Fu R, Tang Y, Li W, et al. Endocannabinoid signaling in the lateral habenula regulates pain and alcohol consumption. *Transl Psychiatry.* 2021;11(1):220. https://doi.org/10.1038/s41398-021-01337-3.
132. Clerke JA, Congiu M, Mameli M. Neuronal adaptations in the lateral habenula during drug withdrawal: Preclinical evidence for addiction therapy. *Neuropharmacology.* 2021;192:108617. https://doi.org/10.1016/j.neuropharm.2021.108617.
133. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci U S A.* 2002;99(12):8394-8399. https://doi.org/10.1073/pnas.082235799.
134. Sloan ME, Gowin JL, Yan J, et al. Severity of alcohol dependence is associated with the fatty acid amide hydrolase Pro129Thr missense variant. *Addict Biol.* 2018;23(1):474-484. https://doi.org/10.1111/adb.12491.
135. Bühler KM, Huertas E, Echeverry-Alzate V, et al. Risky alcohol consumption in young people is associated with the fatty acid amide hydrolase gene polymorphism $C_3 8_5$A and affective rating of drug pictures. *Mol Genet Genomics.* 2014;289(3):279-289. https://doi.org/10.1007/s00438-013-0809-x.
136. Beltramo M, Piomelli D. Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. *NeuroReport.* 2000;11(6):1231-1235. https://doi.org/10.1097/00001756-200004270-00018.
137. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science.* 1997;277(5329):1094-1097. https://doi.org/10.1126/science.277.5329.1094.
138. Hajos N, Kathuria S, Dinh T, Piomelli D, Freund TF. Endocannabinoid transport tightly controls 2-arachidonoyl glycerol actions in the hippocampus: Effects of low temperature and the transport inhibitor AM404. *Eur J Neurosci.* 2004;19(11):2991-2996. https://doi.org/10.1111/j.0953-816X.2004.03433.x.
139. Gianessi CA, Groman SM, Thompson SL, Jiang M, van der Stelt M, Taylor JR. Endocannabinoid contributions to alcohol habits and motivation: Relevance to treatment. *Addict Biol.* 2020;25(3):e12768. https://doi.org/10.1111/adb.12768.
140. Cippitelli A, Bilbao A, Gorriti MA, et al. The anandamide transport inhibitor *AM404* reduces ethanol self-administration. *Eur J Neurosci.* 2007;26(2):476-486. https://doi.org/10.1111/j.1460-9568.2007.05665.x.
141. Koob GF. Theoretical frameworks and mechanistic aspects of alcohol addiction: Alcohol addiction as a reward deficit disorder. *Curr Top Behav Neurosci.* 2013;13:3-30. https://doi.org/10.1007/7854_2011_129.
142. Koob GF. A role for brain stress systems in addiction. *Neuron.* 2008;59(1):11-34. https://doi.org/10.1016/j.neuron.2008.06.012.
143. Mechoulam R, Parker LA. The endocannabinoid system and the brain. *Annu Rev Psychol.* 2013;64:21-47. https://doi.org/10.1146/annurev-psych-113011-143739.
144. Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology.* 2016;41(1):80-102. https://doi.org/10.1038/npp.2015.166.
145. Bedse G, Centanni SW, Winder DG, Patel S. Endocannabinoid signaling in the central amygdala and bed nucleus of the stria terminalis: Implications for the pathophysiology and treatment of alcohol use disorder. *Alcohol Clin Exp Res.* 2019;43(10):2014-2027. https://doi.org/10.1111/acer.14159.
146. Petrie GN, Nastase AS, Aukema RJ, Hill MN. Endocannabinoids, cannabinoids and the regulation of anxiety. *Neuropharmacology.* 2021:108626. https://doi.org/10.1016/j.neuropharm.2021.108626.
147. Tsou K, Brown S, Sañudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience.* 1998;83(2):393-411. https://doi.org/10.1016/s0306-4522(97)00436-3.
148. Puente N, Elezgarai I, Lafourcade M, et al. Localization and function of the cannabinoid CB1 receptor in the anterolateral bed nucleus of the stria terminalis. *PLoS One.* 2010;5(1):e8869. https://doi.org/10.1371/journal.pone.0008869.
149. Hillard CJ, Liu QS. Endocannabinoid signaling in the etiology and treatment of major depressive illness. *Curr Pharm Des.* 2014;20(23):3795-3811. https://doi.org/10.2174/13816128113196660735.
150. Dannon PN, Lowengrub K, Amiaz R, Grunhaus L, Kotler M. Comorbid cannabis use and panic disorder: Short term and long term follow-up study. *Hum Psychopharmacol.* 2004;19(2):97-101. https://doi.org/10.1002/hup.560.
151. Tournier M, Sorbara F, Gindre C, Swendsen JD, Verdoux H. Cannabis use and anxiety in daily life: A naturalistic investigation in a non-clinical population. *Psychiatry Res.* 2003;118(1):1-8. https://doi.org/10.1016/s0165-1781(03)00052-0.
152. Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R. Behavioural and biochemical evidence for interactions between Δ9-tetrahydrocannabinol and nicotine. *Br J Pharmacol.* 2002;135(2):564-578. https://doi.org/10.1038/sj.bjp.0704479.

153. Braida D, Limonta V, Malabarba L, Zani A, Sala M. 5-HT_{1A} receptors are involved in the anxiolytic effect of Δ^9-tetrahydrocannabinol and AM 404, the anandamide transport inhibitor, in Sprague-Dawley rats. *Eur J Pharmacol.* 2007;555(2-3):156-163. https://doi.org/10.1016/j.ejphar.2006.10.038.
154. Rubino T, Sala M, Viganò D, et al. Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral Δ^9-tetrahydrocannabinol in rats. *Neuropsychopharmacology.* 2007;32(9):2036-2045. https://doi.org/10.1038/sj.npp.1301330.
155. Berrendero F, Maldonado R. Involvement of the opioid system in the anxiolytic-like effects induced by Δ^9-tetrahydrocannabinol. *Psychopharmacology (Berl).* 2002;163(1):111-117. https://doi.org/10.1007/s00213-002-1144-9.
156. Rock EM, Limebeer CL, Petrie GN, Williams LA, Mechoulam R, Parker LA. Effect of prior foot shock stress and Δ^9-tetrahydrocannabinol, cannabidiolic acid, and cannabidiol on anxiety-like responding in the light-dark emergence test in rats. *Psychopharmacology (Berl).* 2017;234(14):2207-2217. https://doi.org/10.1007/s00213-017-4626-5.
157. Todd SM, Arnold JC. Neural correlates of interactions between cannabidiol and Δ^9-tetrahydrocannabinol in mice: Implications for medical cannabis. *Br J Pharmacol.* 2016;173(1):53-65. https://doi.org/10.1111/bph.13333.
158. Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: Further evidence for an anxiolytic role for endogenous cannabinoid signaling. *J Pharmacol Exp Ther.* 2006;318(1):304-311. https://doi.org/10.1124/jpet.106.101287.
159. Haller J, Varga B, Ledent C, Freund TF. CB1 cannabinoid receptors mediate anxiolytic effects: Convergent genetic and pharmacological evidence with CB1-specific agents. *Behav Pharmacol.* 2004;15(4):299-304. https://doi.org/10.1097/01.fbp.0000135704.56422.40.
160. Rodríguez de Fonseca F, Rubio P, Menzaghi F, et al. Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J Pharmacol Exp Ther.* 1996;276(1):56-64.
161. Hu SS, Mackie K. Distribution of the endocannabinoid system in the central nervous system. *Handb Exp Pharmacol.* 2015;231:59-93. https://doi.org/10.1007/978-3-319-20825-1_3.
162. Ruehle S, Remmers F, Romo-Parra H, et al. Cannabinoid CB_1 receptor in dorsal telencephalic glutamatergic neurons: Distinctive sufficiency for hippocampus-dependent and amygdala-dependent synaptic and behavioral functions. *J Neurosci.* 2013;33(25):10264-10277. https://doi.org/10.1523/JNEUROSCI.4171-12.2013.
163. Lafenêtre P, Chaouloff F, Marsicano G. Bidirectional regulation of novelty-induced behavioral inhibition by the endocannabinoid system. *Neuropharmacology.* 2009;57(7-8):715-721. https://doi.org/10.1016/j.neuropharm.2009.07.014.
164. Rey AA, Purrio M, Viveros MP, Lutz B. Biphasic effects of cannabinoids in anxiety responses: CB1 and $GABA_B$ receptors in the balance of GABAergic and glutamatergic neurotransmission. *Neuropsychopharmacology.* 2012;37(12):2624-2634. https://doi.org/10.1038/npp.2012.123.
165. Bedse G, Bluett RJ, Patrick TA, et al. Therapeutic endocannabinoid augmentation for mood and anxiety disorders: Comparative profiling of FAAH, MAGL and dual inhibitors. *Transl Psychiatry.* 2018;8(1):92. https://doi.org/10.1038/s41398-018-0141-7.
166. Bluett RJ, Gamble-George JC, Hermanson DJ, Hartley ND, Marnett LJ, Patel S. Central anandamide deficiency predicts stress-induced anxiety: Behavioral reversal through endocannabinoid augmentation. *Transl Psychiatry.* 2014;4:e408. https://doi.org/10.1038/tp.2014.53.
167. Marco EM, Rapino C, Caprioli A, Borsini F, Laviola G, Maccarrone M. Potential therapeutic value of a novel FAAH inhibitor for the treatment of anxiety. *PLoS One.* 2015;10(9):e0137034. https://doi.org/10.1371/journal.pone.0137034.
168. Pavón FJ, Polis IY, Stouffer DG, et al. Selective inhibition of monoacylglycerol lipase is associated with passive coping behavior and attenuation of stress-induced dopamine release in the medial prefrontal cortex. *Neurobiol Stress.* 2021;14:100293. https://doi.org/10.1016/j.ynstr.2021.100293.
169. Moreira FA, Kaiser N, Monory K, Lutz B. Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. *Neuropharmacology.* 2008;54(1):141-150. https://doi.org/10.1016/j.neuropharm.2007.07.005.
170. Naidu PS, Varvel SA, Ahn K, Cravatt BF, Martin BR, Lichtman AH. Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. *Psychopharmacology (Berl).* 2007;192(1):61-70. https://doi.org/10.1007/s00213-006-0689-4.
171. Aliczki M, Balogh Z, Tulogdi A, Haller J. The temporal dynamics of the effects of monoacylglycerol lipase blockade on locomotion, anxiety, and body temperature. *Behav Pharmacol.* 2012;23(4):348-357. https://doi.org/10.1097/FBP.0b013e3283564dfa.
172. Aliczki M, Zelena D, Mikics E, et al. Monoacylglycerol lipase inhibition-induced changes in plasma corticosterone levels, anxiety and locomotor activity in male CD1 mice. *Horm Behav.* 2013;63(5):752-758. https://doi.org/10.1016/j.yhbeh.2013.03.017.
173. Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. *Biol Psychiatry.* 2011;70(5):479-486. https://doi.org/10.1016/j.biopsych.2011.04.022.
174. Sciolino NR, Zhou W, Hohmann AG. Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. *Pharmacol Res.* 2011;64(3):226-234. https://doi.org/10.1016/j.phrs.2011.04.010.
175. Kinsey SG, O'Neal ST, Long JZ, Cravatt BF, Lichtman AH. Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay. *Pharmacol Biochem Behav.* 2011;98(1):21-27. https://doi.org/10.1016/j.pbb.2010.12.002.
176. Sumislawski JJ, Ramikie TS, Patel S. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: A potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. *Neuropsychopharmacology.* 2011;36(13):2750-2761. https://doi.org/10.1038/npp.2011.166.
177. Ciccocioppo R, Economidou D, Cippitelli A, et al. Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: An animal model to study the neurobiology of alcoholism. *Addict Biol.* 2006;11(3-4):339-355. https://doi.org/10.1111/j.1369-1600.2006.00032.x.
178. Gray JM, Vecchiarelli HA, Morena M, et al. Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. *J Neurosci.* 2015;35(9):3879-3892. https://doi.org/10.1523/JNEUROSCI.2737-14.2015.
179. Natividad LA, Steinman MQ, McGinn MA, et al. Impaired hypothalamic feedback dysregulates brain glucocorticoid signaling in genetically-selected Marchigian Sardinian alcohol-preferring rats. *Addict Biol.* 2021;26(3):e12978. https://doi.org/10.1111/adb.12978.
180. Gray JM, Wilson CD, Lee TT, et al. Sustained glucocorticoid exposure recruits cortico-limbic CRH signaling to modulate endocannabinoid function. *Psychoneuroendocrinology.* 2016;66:151-158. https://doi.org/10.1016/j.psyneuen.2016.01.004.
181. Manduca A, Morena M, Campolongo P, et al. Distinct roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in social behavior and emotionality at different developmental ages in rats. *Eur Neuropsychopharmacol.* 2015;25(8):1362-1374. https://doi.org/10.1016/j.euroneuro.2015.04.005.

182. Long JZ, Nomura DK, Vann RE, et al. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc Natl Acad Sci U S A.* 2009;106(48):20270-20275. https://doi.org/10.1073/pnas.0909411106.
183. Schlosburg JE, Kinsey SG, Ignatowska-Jankowska B, et al. Prolonged monoacylglycerol lipase blockade causes equivalent cannabinoid receptor type 1 receptor-mediated adaptations in fatty acid amide hydrolase wild-type and knockout mice. *J Pharmacol Exp Ther.* 2014;350(2):196-204. https://doi.org/10.1124/jpet.114.212753.
184. Khatri D, Laroche G, Grant ML, et al. Acute ethanol inhibition of adult hippocampal neurogenesis involves CB1 cannabinoid receptor signaling. *Alcohol Clin Exp Res.* 2018;42(4):718-726. https://doi.org/10.1111/acer.13608.
185. Shonesy BC, Bluett RJ, Ramikie TS, et al. Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. *Cell Rep.* 2014;9(5):1644-1653. https://doi.org/10.1016/j.celrep.2014.11.001.
186. Jenniches I, Ternes S, Albayram O, et al. Anxiety, stress, and fear response in mice with reduced endocannabinoid levels. *Biol Psychiatry.* 2016;79(10):858-868. https://doi.org/10.1016/j.biopsych.2015.03.033.
187. Bedse G, Hartley ND, Neale E, et al. Functional redundancy between canonical endocannabinoid signaling systems in the modulation of anxiety. *Biol Psychiatry.* 2017;82(7):488-499. https://doi.org/10.1016/j.biopsych.2017.03.002.
188. Winters ND, Bedse G, Astafyev AA, et al. Targeting diacylglycerol lipase reduces alcohol consumption in preclinical models. *J Clin Invest.* 2021;131(17):e146861. https://doi.org/10.1172/JCI146861.
189. Chang JW, Cognetta AB 3rd, Niphakis MJ, Cravatt BF. Proteome-wide reactivity profiling identifies diverse carbamate chemotypes tuned for serine hydrolase inhibition. *ACS Chem Biol.* 2013;8(7):1590-1599. https://doi.org/10.1021/cb400261h.
190. Huggins JP, Smart TS, Langman S, Taylor L, Young T. An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain.* 2012;153(9):1837-1846. https://doi.org/10.1016/j.pain.2012.04.020.
191. Mayo LM, Asratian A, Lindé J, et al. Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: A randomized, controlled experimental medicine trial. *Biol Psychiatry.* 2020;87(6):538-547. https://doi.org/10.1016/j.biopsych.2019.07.034.
192. D'Souza DC, Cortes-Briones J, Creatura G, et al. Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: A double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. *Lancet Psychiatry.* 2019;6(1):35-45. https://doi.org/10.1016/S2215-0366(18)30427-9.
193. Postnov A, Schmidt ME, Pemberton DJ, et al. Fatty acid amide hydrolase inhibition by JNJ-42165279: A multiple-ascending dose and a positron emission tomography study in healthy volunteers. *Clin Transl Sci.* 2018;11(4):397-404. https://doi.org/10.1111/cts.12548.
194. Schmidt ME, Liebowitz MR, Stein MB, et al. The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: A double-blind, randomized, placebo-controlled proof-of-concept study. *Neuropsychopharmacology.* 2021;46(5):1004-1010. https://doi.org/10.1038/s41386-020-00888-1.
195. Wagenlehner FME, van Till JWO, Houbiers JGA, et al. Fatty acid amide hydrolase inhibitor treatment in men with chronic prostatitis/chronic pelvic pain syndrome: An adaptive double-blind, randomized controlled trial. *Urology.* 2017;103:191-197. https://doi.org/10.1016/j.urology.2017.02.029.
196. Takizawa M, Hatta T, Iitsuka H, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of ASP3652, a reversible fatty acid amide hydrolase inhibitor, in healthy, nonelderly, Japanese men and elderly, Japanese men and women: A randomized, double-blind, placebo-controlled, single and multiple oral dose, phase I study. *Clin Ther.* 2020;42(5):906-923. https://doi.org/10.1016/j.clinthera.2020.03.021.
197. Houbiers JGA, van Till JWO, Kaper M, et al. An adaptive randomized clinical trial in interstitial cystitis/bladder pain syndrome evaluating efficacy of ASP3652 and the relationship between disease characteristics and Hunner's lesions. *World J Urol.* 2021;39(6):2065-2071. https://doi.org/10.1007/s00345-020-03372-z.
198. Kerbrat A, Ferre JC, Fillatre P, et al. Acute neurologic disorder from an inhibitor of fatty acid amide hydrolase. *N Engl J Med.* 2016;375(18):1717-1725. https://doi.org/10.1056/NEJMoa1604221.
199. van Esbroeck ACM, Janssen APA, Cognetta AB 3rd, et al. Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science.* 2017;356(6342):1084-1087. https://doi.org/10.1126/science.aaf7497.
200. Deng H, Li W. Monoacylglycerol lipase inhibitors: Modulators for lipid metabolism in cancer malignancy, neurological and metabolic disorders. *Acta Pharm Sin B.* 2020;10(4):582-602. https://doi.org/10.1016/j.apsb.2019.10.006.
201. Holleran KM, Winder DG. Preclinical voluntary drinking models for alcohol abstinence-induced affective disturbances in mice. *Genes Brain Behav.* 2017;16(1):8-14. https://doi.org/10.1111/gbb.12338.
202. Kleczkowska P, Smaga I, Filip M, Bujalska-Zadrozny M. Cannabinoid ligands and alcohol addiction: A promising therapeutic tool or a humbug? *Neurotox Res.* 2016;29(1):173-196. https://doi.org/10.1007/s12640-015-9555-7.
203. Panagis G, Mackey B, Vlachou S. Cannabinoid regulation of brain reward processing with an emphasis on the role of CB_1 receptors: A step back into the future. *Front Psychiatry.* 2014;5:92. https://doi.org/10.3389/fpsyt.2014.00092.
204. Erdozain AM, Callado LF. Involvement of the endocannabinoid system in alcohol dependence: The biochemical, behavioral and genetic evidence. *Drug Alcohol Depend.* 2011;117(2-3):102-110. https://doi.org/10.1016/j.drugalcdep.2011.02.003.
205. Sloan ME, Gowin JL, Ramchandani VA, Hurd YL, Le Foll B. The endocannabinoid system as a target for addiction treatment: Trials and tribulations. *Neuropharmacology.* 2017;124:73-83. https://doi.org/10.1016/j.neuropharm.2017.05.031.
206. McPartland JM, Guy GW, Di Marzo V. Care and feeding of the endocannabinoid system: A systematic review of potential clinical interventions that upregulate the endocannabinoid system. *PLoS One.* 2014;9(3):e89566. https://doi.org/10.1371/journal.pone.0089566.
207. Nomura DK, Morrison BE, Blankman JL, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science.* 2011;334(6057):809-813. https://doi.org/10.1126/science.1209200.
208. Mock ED, Mustafa M, Gunduz-Cinar O, et al. Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. *Nat Chem Biol.* 2020;16(6):667-675. https://doi.org/10.1038/s41589-020-0528-7.
209. Dong A, He K, Dudok B, et al. A fluorescent sensor for spatiotemporally resolved imaging of endocannabinoid dynamics in vivo. *Nat Biotechnol.* 2021. https://doi.org/10.1038/s41587-021-01074-4.
210. Liu P, Hamill TG, Chioda M, et al. Discovery of MK-3168: A PET tracer for imaging brain fatty acid amide hydrolase. *ACS Med Chem Lett.* 2013;4(6):509-513. https://doi.org/10.1021/ml4000996.
211. Hattori Y, Aoyama K, Maeda J, et al. Design, synthesis, and evaluation of (4*R*)-1-{3-[2-(^{18}F)fluoro-4-methylpyridin-3-yl]phenyl}-4-[4-(1,3-thiazol-2-ylcarbonyl)piperazin-1-yl]pyrrolidin-2-one ([^{18}F]T-401) as a novel positron-emission tomography imaging agent for monoacylglycerol lipase. *J Med Chem.* 2019;62(5):2362-2375. https://doi.org/10.1021/acs.jmedchem.8b01576.

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2022;42(1):08 | https://doi.org/10.35946/arcr.v42.1.08 Published: 28 April 2022

SCOPING REVIEW

Simultaneous Alcohol and Marijuana Use Among Young Adults: A Scoping Review of Prevalence, Patterns, Psychosocial Correlates, and Consequences

Christine M. Lee,[1] Brian H. Calhoun,[1] Devon Alisa Abdallah,[1] Jessica A. Blayney,[1] Nicole R. Schultz,[1] Meg Brunner,[2] and Megan E. Patrick[3]

[1]Center for the Study of Health and Risk Behaviors, Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, Washington
[2]Addictions, Drug, and Alcohol Institute, Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, Washington
[3]Institute for Social Research, University of Michigan, Ann Arbor, Michigan

Correspondence
Address correspondence concerning this article to Christine M. Lee, Department of Psychiatry and Behavioral Sciences, University of Washington, Box 357238, Seattle, WA 98195. Email: leecm@u.washington.edu

Acknowledgments
This manuscript preparation was supported by National Institute on Alcohol Abuse and Alcoholism grants R01AA025037, R01AA027496, K99AA028777, and F32AA028667.

Disclosures
The authors declare no competing financial or nonfinancial interests.

BACKGROUND: Alcohol and marijuana are commonly used by young adults, and use of both substances, particularly at the same time, is prevalent among this population. Understanding the prevalence, patterns, correlates, and consequences of simultaneous alcohol and marijuana (SAM) use is important to inform interventions. However, this literature is complicated by myriad terms used to describe SAM use, including use with overlapping effects and same-day co-use.
OBJECTIVES: This scoping review identifies and describes the peer-reviewed literature focused on SAM use by young adults and distinguishes simultaneous use from same-day co-use of alcohol and marijuana. This review also provides a narrative summary of the prevalence of SAM use, patterns of SAM and other substance use, psychosocial correlates, and consequences of SAM use.
ELIGIBILITY CRITERIA: This review is limited to papers written in English and published in peer-reviewed journals between January 2000 and August 2021. It includes papers assessing simultaneous use or same-day co-use of alcohol and marijuana among young adults ages 18 to 30. Review papers, qualitative interviews, experimental lab studies, policy work, toxicology or medical reports, and papers focused on neurological outcomes are excluded.
SOURCES OF EVIDENCE: PubMed, PsycINFO, and Web of Science databases were searched. Databases were selected and the search strategy developed in consultation with an information specialist.
CHARTING METHODS: A data charting form was utilized to specify which information would be extracted from included papers. Eight categories of data were extracted: (1) research questions and hypotheses; (2) sample characteristics; (3) study procedures; (4) definition of SAM use; (5) prevalence of SAM use; (6) patterns of SAM and other substance use; (7) psychosocial correlates of SAM use; and (8) consequences of SAM use.
RESULTS: A total of 1,282 papers were identified through initial search terms. Through double-blind title/abstract screening and full-text review, the review was narrowed to 74 papers that met review inclusion criteria. Review of these papers demonstrated that SAM use was prevalent among young adults, particularly among those who reported heavier quantities and more frequent use of alcohol and marijuana. Enhancement-related motives for use were consistently positively associated with SAM use. SAM use was associated with greater perceived positive and negative consequences of alcohol and/or marijuana use. Inconsistencies in prevalence, patterns, correlates, and consequences were found between studies, which may be due to large variations in measurement of SAM use, populations studied, methodological design (e.g., cross-sectional vs. intensive longitudinal), and the covariates included in models.
CONCLUSIONS: The literature on simultaneous use and same-day co-use of alcohol and marijuana has expanded rapidly. Of the 74 included papers (61 on SAM use; 13 on same-day co-use), 60 papers (47 on SAM use; 13 on same-day co-use) were published within the last 5 years. Future research focusing on the ways in which SAM use confers acute risk, above and beyond the risks associated with separate consumption of alcohol and marijuana, is needed for understanding potential targets for intervention.

KEYWORDS: alcohol; marijuana; cannabis; co-use; simultaneous; review; young adult

Alcohol and marijuana are two of the most commonly used substances among young adults in the United States. In the past year, approximately 82% of young adults ages 19 to 30 reported alcohol use and 42% reported marijuana use.[1] Independently, these two substances are associated with numerous short- and long-term risks and harms.[2-5] Those who use both alcohol and marijuana, and in particular those who use both at the same time so that the effects overlap, experience more negative consequences (e.g., getting hurt, heated arguments, trouble with the law) than do individuals who use the substances separately (e.g., alcohol-only or marijuana-only use) or use on the same day but their effects do not overlap.[6,7] Furthermore, cannabis use disorder and alcohol use disorder often overlap, with more than 86% of individuals with a history of cannabis use disorder also meeting current criteria for alcohol use disorder.[8,9] Thus, understanding alcohol and marijuana use—and more specifically simultaneous use of these substances—is critical for the development of prevention and intervention efforts aimed at reducing consequences during the high-risk developmental period of young adulthood.

Simultaneous alcohol and marijuana (SAM) use is generally defined as using both substances at the same time so that their effects overlap. However, this terminology is not always consistent, and SAM use is sometimes also referred to as same-day use, co-use, or cross-fading, among other terms. In contrast, use of both alcohol and marijuana in general, but not necessarily at the same time or on the same day, is considered concurrent use; this is also sometimes referred to as co-use, polysubstance use, or co-occurring use, among other labels.[7,10] A recent focus in the literature has been on trends in concurrent use, such as how changes in marijuana use are associated with changes in alcohol use, and whether use of the two substances is based on complementary (i.e., rising and falling together) or substitution (i.e., one replaces use of the other) effects. (For reviews, see Guttmannova et al.,[11] Subbaraman,[12] and Risso et al.[13]) Given the variation in the operationalization of SAM use, and the application of often similar or the same terms to SAM use as concurrent use, it can be difficult to synthesize the literature specific to SAM use. Not only is it important to understand associations between alcohol and marijuana use in general, or among people who use both, but there is a need to better understand the prevalence, patterns, correlates, and consequences associated with simultaneous use. This is particularly important among young adults, as SAM use prevalence among this age group has been increasing historically.[14] Recent data suggest that many who use both alcohol and marijuana sometimes use both simultaneously[6,15] and are at the highest risk for engaging in SAM use.[14,16]

Recent acknowledgment of the need to identify situational risk factors has led to the examination of proximal predictors of SAM use, including social contexts. The use of timeline follow-back (an assessment method using a calendar and anchoring dates to obtain substance use estimates with retrospective reports on each day of a given period),[17] and daily and ecological momentary assessments (i.e., repeated assessments of substance use behaviors in real time and natural environments)[18] have provided a finer-grained understanding of patterns, correlates, and consequences at the event level. These repeated-measures methods allow for examination of associations between people (e.g., what distinguishes individuals who engage in SAM use from those who do not) and within people (e.g., what distinguishes situations when SAM use occurs compared to when it does not).

The Current Study

The purpose of the present scoping review was to do a comprehensive search for papers referencing SAM use by young adults and to organize the authors' current understanding around this literature to inform future research and intervention work. To the authors' knowledge, this is the first scoping review of this kind. Given the variability in definitions of SAM use in the extant literature, this review was inclusive of studies that examined use of both alcohol and marijuana on the same day without specifying use at the same time or within a specified time period (i.e., same-day co-use), to allow for greater synthesis of findings across study populations and research designs as well as for comparison of SAM use and same-day co-use. The objective of this review was to summarize research on the prevalence of SAM use, patterns of SAM and other substance use, psychosocial correlates (i.e., motives, norms, situational contexts), and consequences of SAM use. Where appropriate, results from studies utilizing repeated-measures designs to summarize the field's current understanding of situation-level risk are highlighted.

Methods

Protocol and Registration

The protocol was based on the 22-item Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR).[19] The protocol was not preregistered, but it can be obtained upon request from the corresponding author.

Eligibility Criteria

Sources of evidence (i.e., papers) were eligible for inclusion if they (1) were published in peer-reviewed journals between January 2000 and August 2021, (2) were written in English, (3) used human participants in the young adult age range (e.g., ages 18 to 30), and (4) included a focus on or measurement of simultaneous use or same-day co-use of alcohol and marijuana. Papers were excluded if they were review papers,

experimental laboratory research, qualitative research, or if they exclusively evaluated policy. In addition, the criteria were refined to exclude neuroscience studies (however, one was included that discussed patterns of SAM and other substance use) and those in which SAM use was based on toxicology or medical reports. The young adult age-related inclusion criterion was meeting one or more of the following: (1) the majority (51% or more) of the sample was between the ages of 18 and 30; (2) the mean or median age of the sample was between the ages of 18 and 30; (3) participants were in 12th grade or college (even if the age was not provided); or (4) an age range that included ages outside of 18 to 30, but with separate findings provided for young adults ages 18 to 30.

Information Sources and Search Strategy

Electronic databases searched included PubMed, PsycINFO, and Web of Science. The electronic search strategy was developed by the team's information specialist and refined through team discussion (see Table 1). The initial search was performed on February 24, 2021. After removing duplicates, papers identified by the search were entered into a Covidence database, which facilitates the use of PRISMA methodology (see Figure 1). An additional PubMed search without the MEDLINE-limiter "humans" was performed on May 20, 2021, to screen papers included in PubMed but not indexed by MEDLINE (e.g., smaller journals, manuscripts deposited into PubMed Central); a final search was conducted on August 25, 2021, to update search results prior to publication. These additional searches used the same strategy as the initial search and were performed by the team's information specialist.

Table 1. Search Criteria for Each Database

Database	Search Strategy	No. of Results Retrieved
PubMed	Original search: February 2021 ((adolesc* OR teen* OR youth* OR "young adult*" OR "young people*" OR "young person*" OR college* OR "high school*" OR "secondary school*" OR "emerging adult*") AND (alcohol OR drink* OR ethanol) AND (marijuana OR cannabi* OR THC) AND ((cross-fad* OR crossfad*) OR (simultaneous* OR concurr* OR cooccur* OR co-occur* OR co-use*))) AND ((humans[Filter]) AND (English[Filter])) May 2021 search (without the "humans" limit) August 2021 search (without the "humans" limit)	705 4 53
PsycINFO	Original search: February 2021 1. (cross-fad* OR crossfad* OR simultaneous OR concurr* OR cooccur* OR co-occur* OR co-use*) 2. (alcohol OR drinking OR ethanol) AND (marijuana OR cannabi* OR THC) 3. (adolesc* OR teen* OR youth* OR young adult* OR young people* OR college* OR high school* OR secondary school* OR emerging adult*) Limits: Human, English, all journals August 2021 search	700 49
Web of Science	Original search: February 2021 1. TS = (cross-fad* OR crossfad* OR simultaneous OR concurr* OR cooccur* OR co-occur* OR co-use) 2. TS = (alcohol OR drinking OR ethanol) AND ALL = (marijuana OR cannabi* OR THC) 3. TS = (adolesc* OR teen* OR youth* OR young adult* OR young people* OR young person* OR college* OR high school* OR secondary school* OR emerging adult*) Limits: English August 2021 search	706 54

Note: THC, delta-9-tetrahydrocannabinol; TS, topic search.

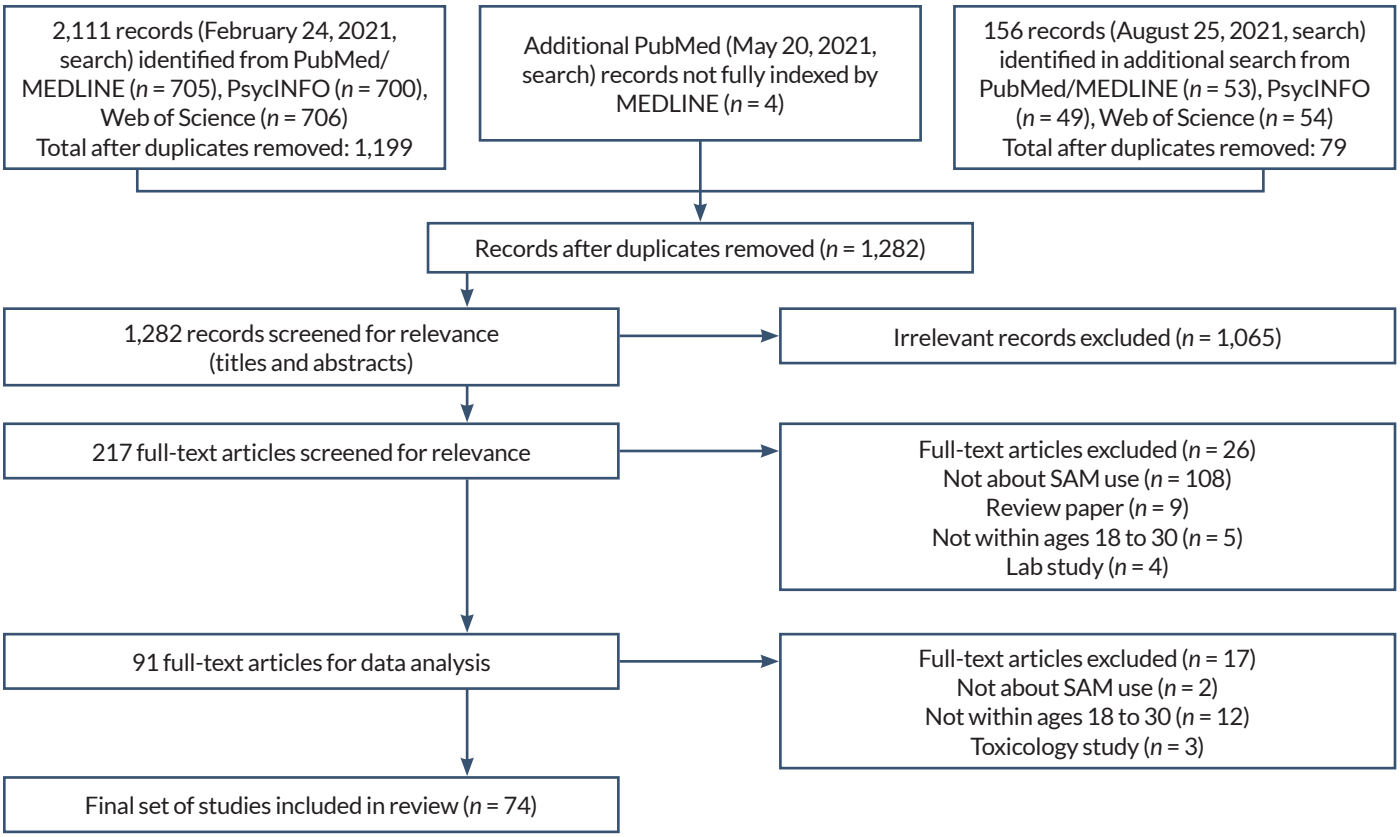

Figure 1. Flow diagram showing literature search and selection of articles. *Note:* SAM, simultaneous alcohol and marijuana.

Selection of Sources of Evidence

Sources of evidence were selected through double-blinded title and abstract screening and full-text review performed in Covidence by four of the authors. The titles and abstracts of all papers identified by the electronic database search were screened by two of the four authors involved at this stage to assess eligibility for inclusion. The full texts of papers not excluded during title and abstract screening were also reviewed by two of the four authors to definitively determine whether papers met all eligibility criteria. Reasons for exclusion decisions were catalogued by Covidence, and disagreements were resolved through discussion.

Data Charting Process and Data Items

Prior to data extraction/charting, the research team developed a data charting form specifying which information would be extracted from included papers. Eight categories of data were extracted: (1) research questions and hypotheses; (2) sample characteristics (i.e., eligibility criteria, age, gender, race/ethnicity) and recruitment procedures; (3) study procedures (i.e., study design, analytic method); (4) SAM use definition; (5) prevalence of SAM use; (6) patterns of SAM and other substance use; (7) psychosocial correlates of SAM use; and (8) consequences of SAM use. Findings generally were extracted only from the text of the results sections to limit assumptions in interpretations of these findings. Information included in tables but not described in the text of the results sections was generally not extracted. The authors met several times to discuss what types of information were to be collected in each category. Papers were divided among the authors, who then extracted the relevant data into the data charting form for each paper. Data items and categories were then divided among authors, and a second author reviewed and revised the extracted data in the data charting form for each data item/category.

Synthesis of Results

Evidence from included papers was grouped into the four areas identified in the review's objectives: (1) prevalence of SAM use, (2) patterns of SAM and other substance use, (3) psychosocial correlates, and (4) consequences of SAM use. Results are presented in narrative format. Some papers provided evidence in more than one area of focus and are included in more than one subsection of the results. Other papers that did not clearly specify SAM use (e.g., those that assessed a broader range of polysubstance use that included illicit drugs such as cocaine, 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy), or psilocybin mushrooms in addition to alcohol and marijuana) or did not directly test associations within the review's objectives (e.g., papers in which SAM use was tested as a moderator) are retained in Appendix 1 but are not described in the Results section.

Results

Selection of Sources of Evidence

As shown in the PRISMA diagram in Figure 1, the initial electronic database searches conducted in February 2021 identified 2,111 records (1,199 nonduplicate papers) related to SAM use or same-day co-use that were written in English and published in peer-reviewed journals between January 2000 and February 2021. After abstract and title screening, 179 papers were deemed eligible for full-text review. After full-text review, 55 papers met all inclusion criteria and were included in the scoping review. A second PubMed search was conducted in May 2021 yielding four additional records (no duplicated papers), all of which were deemed eligible for full-text review and three of which are included in the scoping review. A third search of all three databases in August 2021 identified 156 records (79 nonduplicate papers) published since the date of the initial search (February 2021), of which 34 were deemed eligible for full-text review and 16 met all inclusion criteria and are included in the scoping review. In summary, 1,282 nonduplicate papers related to SAM use or same-day co-use were identified, 217 papers underwent full-text review, and a total of 74 papers are included in this scoping review.

Characteristics of Sources of Evidence

Appendix 1 provides a list of all 74 papers identified in the final search for relevance for this scoping review. The appendix includes each paper's methodological design, population, age range, sample size, SAM definition, and whether it is included in the Results section of this review in reference to prevalence, patterns, correlates, and/or consequences of SAM use.

To capture all relevant papers, the authors started the search with inclusive terms for young adult and concurrent or simultaneous alcohol and marijuana use and then systematically reviewed these papers for relevance to SAM use or same-day co-use. This process resulted in a set of papers that was more focused, but continued to vary widely in sample, methods, and measures. The time frames (e.g., yesterday, past month, past 3 months, past year) and response options (e.g., dichotomous, ordinal) of SAM use measures differed between papers. Of the papers included in this review, use was operationalized into four categories based on whether alcohol and marijuana use were specified as occurring simultaneously or overlapping or within different dimensions of same-day use. The categories include using alcohol and marijuana:

- At the same time or together so that their effects overlapped (n = 27 papers)
- On the same day within a specified time period (e.g., within 3 hours of each other; n = 9 papers)
- At the same time or together without specifying that their effects overlapped or at the same event or occasion without specifying overlapping effects of use within a specified time period (e.g., at the last party attended, during the current night out; n = 25 papers)
- On the same day without specifying that they were used together or within a specified time period (n = 13 papers)

After careful discussion, the authors categorized SAM use as being inclusive of the first three categories. The fourth category was considered "same-day co-use"—rather than SAM use—because it could not be determined whether alcohol and marijuana use were overlapping or used in relatively close timing with each other. The same-day co-use category was included in this review given varying definitions of SAM use to sometimes include these types of definitions. By inclusion, it may help specify differences in findings. Therefore, of the 74 included papers, 61 were categorized as SAM use and 13 as same-day co-use.

Of the 74 papers, 36 analyzed cross-sectional data and 38 analyzed longitudinal data. Of the papers reporting longitudinal data, nine used data from panel studies with various follow-up intervals, and 22 used data from daily or ecological momentary assessment studies that allowed for testing between- and within-person associations. The remaining seven papers used data collected via the timeline follow-back method, in which participants reported their substance use at a single time point, but the assessment referenced a past series of days (e.g., past month), resulting in a series of day- or occasion-level substance use reports.

Of the 74 included papers, 45 (61%) focused exclusively on young adults ages 18 to 30; 18 (24%) used samples including individuals on the younger end of the age range (e.g., 12th-grade students) or included both late adolescents and young adults; and 11 (15%) included a larger age range of adults, with either a majority of the sample in the young adult age group or estimates stratified by age ranges.

Prevalence of SAM Use

There were eight papers from nationally representative U.S. samples. Six were from the Monitoring the Future (MTF) study, and two were from the National Alcohol Survey. Estimates based on MTF data indicated that 20% to 25% of 12th-grade students (modal age 18) reported past-year SAM use, both when averaging across longer time periods (e.g., 1976–2011) and shorter, more recent periods (e.g., 2007–2016).[15,20-22] An estimated 6% to 7% of 12th-grade students engaged in SAM use most or all of the time.[20,21] Similar findings were noted at later ages (e.g., modal ages 19 or 20 through 29 or 30) in papers following MTF participants longitudinally.[14,16] Estimates based on National Alcohol Survey data found that approximately 15% of young adults ages 18 to 29 who reported drinking in the past year also reported past-year SAM use in data from 2000, 2005, and 2010.[6,23]

Historical trends

Three papers, all from MTF, reported on historical trends in SAM use over sufficiently long periods of time with nationally representative U.S. samples.[14,20,21] Overall trends in SAM use were closely tied to trends in marijuana use and alcohol use.[14,20,21] Among 12th-grade students who reported marijuana use, SAM use trends were highly correlated with alcohol use.[21] Correspondingly, among young adults who reported alcohol use, SAM use trends were highly correlated with trends in marijuana use prevalence.[14] Generally, the prevalence of past-year SAM use among 12th-grade students was highest in the late 1970s, decreased throughout the 1980s and early 1990s, increased during the mid- and late 1990s, and was relatively stable from the late 1990s until 2007, when a slight increase was observed through 2011.[20] Among young adults who used alcohol, SAM use trends varied by age.[14] For those ages 19 to 28, SAM use prevalence generally decreased from the mid-1970s through the early to mid-1990s, but prevalence was stable for those ages 29 or 30.[14] From the early to mid-1990s through 2011, trends continued to vary by age, ranging from an increase through the mid-2000s followed by no significant change for those ages 19 or 20, to generally consistent increases in use for those ages 21 to 26, to stable use prevalence for those ages 27 or 28.[14]

Demographic characteristics

Most papers examining gender and/or sex differences in SAM use, including those using nonrepresentative samples, found that a greater proportion of males than females engaged in SAM use.[15,23-28] One paper also found that males consumed greater amounts of alcohol and were high for greater lengths of time on SAM use days than females.[29] Fewer papers examined race/ethnicity differences. Those that did generally found that White young adults, in comparison to young adults of other racial/ethnic groups, were more likely to engage in SAM use, did so more frequently, and tended to consume greater quantities of alcohol and marijuana when engaging in SAM use.[15,16,21] However, these findings were not consistent, and some depended on whether analyses were bivariate or multivariate. Only one paper examined age differences in SAM use during young adulthood with rigor.[14] This paper used MTF data to estimate SAM use prevalence among young adults who drank alcohol at six modal ages and found SAM use prevalence was highest between ages 19 and 22 at approximately 30%, decreased throughout the twenties, and reached 19% at modal age 29 or 30. A few papers examined differences in SAM use between full-time 4-year college students and non–college students.[16,30] One paper found the likelihood of SAM use was higher for college students not living with their parents relative to those living with their parents.[16] Another paper found that the within-person association between alcohol and marijuana use was weaker for college students compared to young adults not in college.[30]

Patterns of SAM and Other Substance Use

SAM use appears to be most common among individuals who use alcohol, marijuana, or illicit drugs more frequently and in greater amounts. Many papers found SAM use was greater among those who engage in heavier drinking and marijuana use.[16,20-24,28,31-38] For instance, one paper found that SAM use was most prevalent among those using four or more modes of cannabis administration (e.g., joint, bong, vape, edibles).[39] Another found that individuals who engaged in more frequent SAM use had a greater likelihood of any illicit drug use (not including marijuana).[21]

Six papers using mixture models (e.g., latent class/profile analysis) to examine patterns of SAM use with other substance use found similar results. Generally, latent classes with high probabilities of SAM use also had high probabilities of other risky substance use behaviors (e.g., using alcohol and marijuana with greater frequency or in greater quantities, experimentation with illicit drugs).[15,40-42] In three of these papers, SAM use distinguished one or more latent classes of individuals who use substances from others.[15,40,41] The probability of using tobacco and other drugs (i.e., other than alcohol, marijuana, tobacco) was 50% or greater in each profile associated with SAM use.[43] One paper using mixture models was an exception in that it found that the latent class with the lowest probabilities of substance use reported the highest past-year frequencies of SAM use.[44] However, this paper's findings may be biased due to its eligibility criteria (e.g., past-year alcohol, marijuana, and tobacco use), sampling method (i.e., convenience sampling from Craigslist), and sample characteristics (i.e., 89% male; 86% White).

Papers examining daily associations of SAM use or same-day co-use with alcohol and marijuana in terms of consumption and intoxication have produced inconclusive findings. Regarding daily associations between SAM use and alcohol intake, one paper found that young adults consumed more alcohol on SAM use days relative to alcohol-only use days,[32] whereas another paper found no differences in alcohol (number of drinks) or marijuana (number of hits) consumption on SAM use days relative to alcohol- and marijuana-only use days, respectively.[45] For same-day co-use, several papers found that more alcohol was consumed on days marijuana was also used relative to days that only alcohol was used.[46-48] Between-person findings in these papers provided some evidence that greater average alcohol intake was associated with more frequent SAM use[32] and less frequent same-day co-use.[46,47]

Regarding daily associations between SAM use and intoxication, one paper found that young adults reported greater subjective intoxication on SAM use days as compared to both alcohol-only and marijuana-only use days,[49] whereas another found no differences in level of subjective intoxication on SAM use days as compared to both alcohol-only and marijuana-only use days.[45] Some evidence suggests that SAM use may moderate associations between alcohol and marijuana intake and subjective intoxication such that these associations are weaker

on SAM use days relative to alcohol-only and marijuana-only use days, respectively.[49] For same-day co-use, one paper found that estimated blood alcohol concentrations were higher on days when both alcohol and marijuana were used relative to days when only alcohol was used.[46] Another paper examining same-day co-use found that young adults tended to drink less alcohol on days when marijuana was used before alcohol.[50]

Psychosocial Correlates of SAM Use

Situational and peer context

Eight papers examined contexts associated with SAM use.[21,25,31,38,51-54] Overall, context was an important correlate associated with SAM use across samples (community, treatment seeking) and designs (cross-sectional, event-level). However, findings on specific settings were mixed. Among papers using cross-sectional data, SAM use was significantly less likely to occur in bars and restaurants compared to outdoor and public locations (e.g., park, beach).[52] However, the likelihood of SAM use was higher in settings in which more people were perceived to be intoxicated,[52] and individuals had increased odds of SAM use if they engaged in more alcohol and/or marijuana use in certain settings (e.g., park).[21] In contrast, among a sample of treatment-seeking adults in Canada, SAM use was more likely than marijuana use alone to occur across settings and social compositions, including at home (alone or with friends), at work/school (alone or with friends), with strangers, at bars or taverns, and when driving a car.[25]

Findings from papers using daily or ecological momentary assessment data were also mixed. Associations between contexts and SAM use seemed to differ based on participants' ages as well as whether the comparison day was alcohol-only or marijuana-only use.[51,54] One paper found that college students were more likely to engage in SAM use—compared to alcohol-only and marijuana-only use—at a friend's place.[54] These students were also more likely to engage in SAM use at parties and less likely to engage in SAM use at a bar or restaurant relative to alcohol use only.[54] This paper also found that college students were more likely to engage in SAM use relative to marijuana use only in contexts with greater numbers of people.[54] Another paper found that associations between SAM use and contexts differed between young adults under age 21 and those age 21 and older.[51] For those under age 21, SAM use was more likely to occur at home than alcohol-only use, but odds of SAM use across other physical contexts did not differ from alcohol-only use. For those age 21 and older, SAM use, compared to alcohol-only use, was more likely to occur at a friend's house or outdoors and less likely to occur in a bar or restaurant. For those age 21 and older but not those under age 21, SAM use was less likely than alcohol-only use to occur when young adults were alone.[51]

Two papers using longitudinal data examined the relationship between social networks and SAM use. In a paper on the role of friends among college students, data collected over two semesters showed that having a greater proportion of friends who used alcohol or marijuana was related to greater likelihood of simultaneous use compared to concurrent use.[31] In an investigation of how changes from early to late adolescence were associated with SAM use in young adulthood, time with peers using alcohol and marijuana in sixth or seventh grade was predictive of greater likelihood of SAM use in young adulthood (mean age = 20.7).[53] Similarly, greater alcohol and marijuana use by a sibling or an important adult during adolescence was associated with SAM use in young adulthood, although family effects were no longer significant when all domains (individual, peer, family, neighborhood) were included.

Motives for use

A total of seven papers included measures of motives in relation to SAM use or same-day co-use.[21,25,55-59] Across samples, designs, and measures, motives (particularly SAM-specific motives) were found to be an important correlate of SAM use. Two papers (one using cross-sectional data and one using longitudinal data) described the factor structure and validity of four-factor SAM-specific motives measures, including motives for conformity, positive effects, calm/coping, and social.[55,56] The subscales from these SAM-specific motives measures were associated with the frequency of SAM use in the past month[55] and the past 3 months[56] after controlling for alcohol- and marijuana-specific motives.

Three papers utilized daily methods to assess the associations between motives and SAM use or same-day co-use among community samples.[57-59] In a paper assessing both cross-fading motives (i.e., use of alcohol and marijuana at the same time to enhance the positive effects of alcohol or marijuana) and general substance use motives across SAM use occasions, greater cross-fading motives were associated with alcohol use outcomes at the between- and within-person level.[58] Further, enhancement, social, and coping motives were positively associated with alcohol and marijuana use at the within-person level, and general enhancement and coping motives were associated with greater alcohol and marijuana use at the between-person level. When examining general or substance-specific motives, elevated enhancement and coping motives on alcohol use occasions and social motives on marijuana use occasions were associated with a greater likelihood of SAM use at the between-person level.[59] Within-person, elevated conformity, enhancement, and coping motives on alcohol use occasions, as well as social, conformity, and coping motives on marijuana use occasions, were associated with a greater likelihood of SAM use. Finally, compared to days when only marijuana was used, same-day co-use of alcohol and marijuana was associated with elevated marijuana-related enhancement and social motives.[57] Together, these findings show that enhancement motives emerge as an important correlate of SAM use, but other motives (coping, social, conformity) have mixed findings.

Finally, two papers using cross-sectional data examined the "reasons"[21] for and "functions"[25] of SAM use. Similar to the paper

on cross-fading motives,[58] among a national sample of 12th-grade students, using alcohol to increase the effects of another drug had a stronger association with frequency of SAM use than other alcohol-related motives for use.[21] Finally, compared to marijuana use only, SAM use was more likely to occur across all functions assessed, with the greatest odds occurring for self-medication reasons (e.g., "to calm myself down") among treatment-seeking individuals in Canada.[25]

Social norms

Two papers using cross-sectional data found perceived descriptive norms (e.g., perceptions of prevalence and/or quantity of peer substance use) and SAM use frequency were positively associated in samples of college students[60] and community young adults.[35] Further, both papers found that individuals who engaged in SAM use, compared to individuals who used only alcohol[35] and individuals who used alcohol or marijuana but did not engage in SAM use,[60] endorsed greater descriptive norms of their friends' and/or peers' substance use, as measured by the perceived number of drinks in a typical week[35] or the percentage of friends and peers who engaged in SAM use at least monthly.[60]

Expectancies and perceived risk

Two papers included information related to outcome expectancies for alcohol use[52] and SAM use.[53] In one paper, cross-sectional research found that negative expectancies for alcohol-related outcomes were associated with decreased odds of SAM use, but positive expectancies were not associated with odds of SAM use.[52] SAM-specific expectancies were not assessed. In contrast, a longitudinal study examining changes from early to late adolescence found that increases in positive expectancies of SAM use during late adolescence were predictive of SAM use in young adulthood.[53]

Two papers included perceived risk of SAM use. One paper using daily assessment data from a community sample of young adults found that SAM use was especially likely to occur among those with a lower perceived risk of SAM use.[30] Another study using cross-sectional data found that individuals who engaged in heavier alcohol and marijuana use were more likely to have experienced cross-fading (i.e., intoxication from alcohol and marijuana at the same time) and perceived cross-fading as more desirable and less risky.[61]

Other psychosocial or cognitive factors

A cross-sectional study examining behavioral economic demand indices found that individuals who engaged in SAM use exhibited greater overall expenditures on alcohol compared to individuals who used alcohol and marijuana concurrently; moreover, individuals who engaged in SAM use were less sensitive to alcohol price increases than were individuals who used both substances concurrently.[62] In additional papers, SAM use was positively associated with sensation seeking among a community sample who engaged in past-year SAM use,[35] was not associated with working memory in a community sample,[63] and was less likely to occur on days on which college students used certain adaptive emotion regulation strategies (i.e., reappraisal, problem-solving).[64] In addition, SAM use was positively associated with depressive symptoms cross-sectionally in a community sample[52] and in a national sample of young adults.[23] Compared to alcohol-only use, SAM use and SAM use frequency were associated with higher levels of psychosis, oppositional defiant disorder, and conduct disorder in a community sample of young adults.[28] Another paper found that young adults who reported more depressive symptoms across 2 years also reported more frequent SAM use; furthermore, during months with more depressive symptoms, young adults engaged in more SAM use compared to months when they used alcohol only (levels of depressive symptoms did not differ across months with SAM use compared to neither alcohol nor marijuana or concurrent use).[65] Further, SAM use was positively associated with likelihood of alcohol dependence.[23] Among a Swiss population that engaged in same-day co-use of alcohol and marijuana, symptoms of alcohol use disorder and cannabis use disorder appeared to be associated with distinct clusters of symptoms rather than overlapping disorders.[66]

Consequences Associated With SAM Use

Negative consequences of SAM use

Thirty-three papers (14 cross-sectional, five longitudinal, and 14 event-level) examined associations between SAM use or same-day co-use and the negative consequences of use. The measurement of negative consequences in these papers largely centered around alcohol, and papers varied widely in their definition and measurement of consequences. This assessment typically involved pooling items from existing alcohol and/or marijuana consequence measures and modifying the instructions (e.g., "Below is a list of things that sometimes happen to people either during or after they have been drinking alcohol or using marijuana."[24]). Among most cross-sectional and longitudinal papers,[6,23,24,28,35,36,38,55,56,60,65,67,68] evidence consistently suggested a positive association between SAM use or same-day co-use and number of negative consequences experienced, even after controlling for demographics, impulsivity, delinquency, motives, alcohol use, and/or marijuana use. Of these papers, half focused on comparing individuals who engage in SAM use to individuals who use both substances concurrently or individuals who use alcohol only,[6,24,35,36,38,68] whereas the remaining focused on SAM use frequency as a predictor of consequences.[23,28,55,56,60,67] In both college and community samples, individuals who engaged in SAM use reported a greater number of negative consequences relative to those who used alcohol only,[24,35,36] though findings were mixed when comparing individuals who engaged in SAM use with those who used concurrently.[24,36,38] Papers on SAM use frequency showed a similar pattern, with more frequent SAM use associated with greater negative consequences.[55,56,60]

Others have found that using only specific marijuana–alcohol combinations, such as combining only leaf or concentrate marijuana products with beer, during the same occasion may actually decrease the odds of negative SAM-related consequences relative to using multiple marijuana products (e.g., leaf, concentrate, edible) and/or multiple alcohol products (e.g., beer, wine, liquor).[33] Interestingly, ordering effects (i.e., using alcohol before marijuana vs. using marijuana before alcohol) on same-day co-use occasions were not associated with the number of negative consequences.[49,50] Days with heavy episodic drinking (HED; i.e., 4+/5+ drinks for women/men) and marijuana use were associated with increased risk for consequences relative to days in which young adults engaged in non-HED drinking, non-HED drinking and marijuana use, and/or marijuana-only use.[49,69] Notably, non-HED drinking occasions may not differ from non-HED and marijuana use occasions or marijuana-only occasions with regard to alcohol consequences.[69]

Although most papers examined consequences broadly, a subset of papers investigated specific consequence types, including academic, cognitive, social, sexual, aggression, and sleep-related.[6,23,24,36,65,67,68,70-72] Compared to those who used alcohol only, individuals who engaged in SAM use were at higher risk across consequence types,[6,23,24,36] including alcohol-related harms (e.g., problems with relationships, finances, work, or health).[6] Fewer papers included individuals who used alcohol and marijuana concurrently but did not engage in SAM use, as a comparison.[6,24,36] Among those papers, individuals who engaged in SAM use reported more blackouts, risky driving, and negative academic consequences,[24,36] but differences in social consequences were mixed.[6,36] This elevated risk—both broadly and for specific types of consequences—appeared to be a function of high-intensity drinking (i.e., drinking more than twice the binge drinking threshold)[68] and more frequent simultaneous use.[24] Other factors, such as SAM-specific norms and motives, also were found to increase negative consequences,[55,56,60,73] including those specific to marijuana use[55] and SAM use.[56] Interestingly, young adults tended to attribute the consequences they experience more to alcohol use than to SAM use.[24]

Among the papers using daily assessments, both between- and within-person effects of SAM use on negative consequences have emerged.[32,33,45,49,58,74,75] Although most of the papers in this area assessed consequences specific to substance use type (i.e., alcohol, marijuana, SAM), some combined consequences across substances (e.g., total substance-related consequences).[45,49] At the between-person level, young adults with stronger cross-fading motives on average reported more negative alcohol consequences, but not more negative marijuana consequences.[58] At the within-person level, the effect of SAM use on negative consequences was more pronounced. Among a sample of youth and young adults, SAM use (relative to alcohol-only use) at the last party attended was associated with greater odds of negative consequences (e.g., getting in a fight, having unprotected sex, experiencing forced sex, getting into a car crash, getting in trouble with parents, having a hangover).[74] Other papers linked SAM use to greater consequences relative to alcohol-only or marijuana-only use occasions.[45] Still, not all papers found a link between same-day co-use and consequences after controlling for alcohol and/or marijuana use.[29,32,67,75] For example, among college men, there was no evidence of same-day co-use increasing the likelihood of interpersonal conflict above and beyond alcohol or marijuana use.[67]

SAM use and risky driving

Eleven papers (seven cross-sectional, one longitudinal, and three daily assessment) examined SAM use and risky driving. In these papers, risky driving was typically assessed as a single item (e.g., substance-involved driving, being stopped by the police, tickets/warnings/accidents), with the exception of one community study that incorporated a multiple-item measure of driving risk.[76] Among college and community samples, individuals who engaged in SAM use were more likely to report risky driving compared to those who used alcohol only,[6,20,24,76] those who used marijuana only,[76] or those who co-used alcohol and marijuana.[36] Relative to individuals who only used marijuana or only drank alcohol, individuals who engaged in SAM use endorsed lower risk perceptions for substance-involved driving.[76] In a paper on young adults sampled when leaving a college district bar, 45% of participants who engaged in SAM use that night reported intending to drive after leaving the bar relative to 29% of those who used alcohol only.[77] Findings linking SAM use with a greater likelihood of riding with an intoxicated driver have been mixed, as one paper found evidence supporting this association[78] and another did not.[34] A third paper found evidence indicating that same-day co-use was associated with greater odds of riding with an intoxicated driver in comparison to alcohol-only days.[79]

Perceived or subjective positive effects or consequences

Four papers using daily assessments explored associations between SAM use and its perceived or subjective positive effects or consequences (e.g., feeling relaxed, social, or buzzed).[29,32,45,58] Across these papers, the measurement of positive consequences centered around alcohol,[29,32,58] marijuana,[29,58] or substance use more broadly.[45] Findings revealed a positive association between SAM use days and perceived positive consequences of alcohol[32] and/or substance use,[45] such that more positive consequences tended to be reported on SAM use days relative to alcohol-only[32] and marijuana-only days.[45] Notably, these effects persisted even after controlling for other relevant factors such as demographics, motives, weekend day, alcohol use, and/or marijuana use. A recent paper found no significant differences in average daily counts of perceived positive consequences between planned and unplanned SAM use days.[29] When considering motives, one paper found that higher cross-fading motives in general were associated with greater perceived positive consequences from alcohol and marijuana; in addition, SAM use days with higher cross-fading motives were associated with greater perceived positive consequences of alcohol.[58]

Discussion

The search identified 74 papers eligible for inclusion in this scoping review on four broad topics relevant to SAM use and same-day co-use by young adults. The four areas reviewed (i.e., prevalence of SAM use, patterns of SAM and other substance use, psychosocial correlates, and consequences of SAM use) elucidate information relevant for the field.

The literature on young adult SAM use is quickly growing. Of the 74 papers (61 on SAM use, 13 on same-day co-use) included in this review, 60 papers (47 on SAM use; 13 on same-day co-use) were published within the last 5 years (since 2017). However, the number of papers within each topic area was fairly limited, with the exception of consequences. Findings suggest that SAM use is prevalent and associated with negative consequences and perceived positive consequences. Review of the papers using nationally representative samples suggests that up to approximately one-quarter of young adults reported SAM use in the prior year,[15,20-22] with a higher prevalence during the transition to young adulthood (i.e., ages 19 to 22).[14] Two papers indicated 15% of young adults (ages 18 to 29) who drink engage in SAM use;[6,23] however, these two studies were conducted prior to the legalization of nonmedical use of marijuana, which started in 2012 in Washington and Colorado and extended to at least 18 states and the District of Columbia by 2021. More recent findings from nationally representative samples suggest that marijuana use and concurrent use of alcohol and marijuana have been increasing steadily.[10] Continued investigation of SAM prevalence in representative samples with data post-2012 is needed, including examination of longitudinal time trends. Although this review focuses on trends from representative samples, individual papers often report higher rates of SAM use when the samples are more specific to those who use alcohol and/or marijuana; one paper found that almost 75% of college students who reported past-year use of alcohol and marijuana engaged in SAM use in the past year,[60] further demonstrating SAM use as a high-risk and prevalent behavior.

There is strong evidence across numerous papers to suggest that engaging in SAM use is common among individuals who engage in heavier and more frequent alcohol and marijuana use, including those who also use illicit substances.[16,20-24,28,31-38] Findings from papers with different designs and analytic techniques consistently show that patterns of alcohol, marijuana, and other substance use distinguish those who engage in SAM use from other patterns of use. However, the evidence is less conclusive regarding the predictors and implications of SAM use for alcohol and marijuana use from event-level studies. The lack of consistent findings at the situation level is likely due, at least in part, to great variation in the eligibility criteria of samples (i.e., based on any use of alcohol, marijuana, or both, or use of either or both at particular levels), differences in the measurement and modeling of SAM use (e.g., comparing SAM days to alcohol-only days, marijuana-only days, or co-use days), and the presence or absence of covariates. Additional research is needed on the types of people and the types of situations that are associated with SAM use and consequences, with particular attention paid to the extent to which findings may or may not be generalizable.

Consistent, strong evidence was found across papers demonstrating associations between SAM use or same-day co-use with negative consequences (typically focused on consequences from alcohol use, but also marijuana or combined substance use),[6,23,24,35,36,55,56,60,67] as well as several other papers documenting associations between SAM use or same-day co-use with mental health and driving risks.[6,20,24,36,76] These effects were often present even after controlling for relevant demographics, alcohol use, and/or marijuana use. Most of the papers assessed the number of consequences reported, with little consistency in the measurement of consequences; fewer papers focused on specific harms. To inform interventions, further understanding of the impacts of SAM use on various aspects of functioning is needed as well as how young adults evaluate these consequences.

Only four papers examined perceived positive consequences associated with SAM use, and participants generally reported more positive consequences on SAM use occasions than alcohol-only or marijuana-only occasions.[29,32,45,58] The theoretical and clinical importance of understanding the perceived positive effects of SAM use may be critical to informing interventions aimed at motivations and expectations related to SAM use. For example, research on alcohol expectancies and consequences has found that young adults perceive some expectancies and consequences as positive or neutral, despite these traditionally being included on measures of negative outcomes (e.g., hangovers).[80,81] There is also emerging evidence that individuals have specific motives for SAM use and that these motives are associated with increased risk of SAM use[58,59,82] and negative consequences in daily assessment studies.[58] Across these papers, enhancement-related motives, including to get cross-faded,[58] were consistently associated with SAM-related behaviors. Surprisingly, only two papers examined social norms related to SAM use,[35,60] despite the large focus on young adult social norms in the alcohol literature.[83]

The authors identified several considerations in interpreting the findings from this review. First, many of the papers reviewed included nonrepresentative samples; thus, it is important to consider inclusion criteria and sample characteristics across papers (see Appendix 1). Sample selection is important for considering the findings, particularly for daily assessment studies, which often use higher-risk samples currently engaging in SAM use. Second, it is important to consider study design and whether or what comparisons are being made to SAM use (e.g., SAM use vs. alcohol-only, marijuana-only, co-use, or non–substance use occasions), particularly when examining effects or negative consequences resulting from SAM use. The question at hand in these studies is determining whether SAM use effects

are "worse" than effects on other use days. Often these studies control for the amount of alcohol and/or marijuana and assume the effect of SAM use is multiplicative. That is, controlling for the amount of use is implicitly testing whether, for example, having seven standard drinks and spending 4 hours high from marijuana leads to greater consequences when this substance use overlaps than if it occurs separately. This analytic design leads to a strict test of the impacts or effects of SAM use, and implicit assumptions of these models often are not discussed. Specifically, although research designs that answer questions about between-person effects are important for determining who may be at risk, the focus on between-person differences does not consider why or when risk for or consequences of SAM use might be greater in an individual's typical day-to-day experience. Conversely, comparisons from daily assessment studies are less universal because the samples are often highly selective. Together, these findings highlight the need for clarity in the descriptions of measures and methods used and the relative benefits and limitations of study designs.

The authors identified some measurement considerations. First, the majority of papers used a dichotomous indicator of any versus no SAM use, which fails to capture the intensity of use of alcohol and/or marijuana. Future studies should include more nuanced measures of SAM use to model this heterogeneity. It is particularly important to specify how SAM use is operationalized in each study to compare results. For example, SAM use that is defined as alcohol and marijuana use that is overlapping or within the same time frame is different than same-day co-use of alcohol and marijuana; different effects may be observed, and there would be different hypothesized mechanisms for risks. As mentioned in the introduction, the terminology for these behaviors varies across studies, which makes synthesizing results challenging. The authors of this review recommend that all authors clearly define the constructs used in their research, while reserving the use of the "simultaneous alcohol and marijuana (SAM) use" terminology for behavior strictly defined as the use of alcohol and marijuana at the same time so that their effects overlap.

Second, consistent with literature related to marijuana use, most studies in this review did not include measurement of marijuana potency or quantity consumed. Unlike alcohol, there is no standard unit measure of marijuana, which is further complicated by differing delta-9-tetrahydrocannabinol (THC) potency and modes of use. Future research should try to include more consistent and nuanced measurement of marijuana use; in fact, the National Institute on Drug Abuse is recommending that researchers utilize a standard THC unit in human subjects research when applicable.[84,85] Further, papers should be reviewed in light of the context in which the data were collected; for example, increases in THC content over time, particularly in states where nonmedical use of marijuana is legal, may confound issues related to SAM use and effects of use. Future research needs more nuanced models and measurements to assess main and synergistic effects of the two substances, including how variations in SAM use may lead to increasing consequences and ultimately to cannabis use disorder and/or alcohol use disorder. Although other polysubstance use is not reviewed here, some studies did include this and suggest that SAM use is an early indicator of simultaneous use with illicit substances.[42]

Prevention/Clinical Implications

Given that individuals who engage in SAM use tend to use alcohol and marijuana more heavily and more frequently, prevention efforts aimed at identifying these individuals are greatly needed, particularly during young adulthood. Notably, once individuals who engage in SAM use are identified, it will be important to determine whether current empirically supported strategies for reducing alcohol use (e.g., brief motivational interventions, personalized feedback interventions)[86] also reduce SAM use. However, there is little evidence that these interventions have a secondary impact on marijuana use,[7,87] although research in this area is limited. Further, it is unclear if stand-alone marijuana interventions (though there are fewer empirically supported stand-alone interventions for young adults compared to alcohol interventions)[88,89] have a secondary effect on alcohol or SAM use. Few interventions for SAM use, particularly for young adults, have been conducted and have yielded limited success.[90] For example, a motivational intervention focused on emerging adult themes (e.g., identity exploration, instability, self-focus, feeling in-between, a sense of possibilities) had no effect on SAM use days,[91] while a brief motivational intervention with adults visiting the emergency department showed reductions in SAM use days.[92] Given these mixed findings, the authors of this review encourage more research, first, to better understand the mechanisms by which SAM use may lead to risk, in order to identify the most appropriate intervention targets. Currently, motives for use (e.g., enhancement, cross-fading) as well as social norms may be good candidates for inclusion in interventions. Young adults may self-select into social groups (e.g., higher proportion of individuals who engage in SAM use) or contexts (e.g., private spaces, outdoor locations) that increase the odds of SAM use. At the situation level, use of protective strategies (e.g., limiting alcohol use before marijuana use, having a designated driver) may help reduce consequences on SAM use occasions, including substance-involved driving.

Limitations of Review

This review should be read within the context of certain caveats, including search terms, databases used, and the inclusion/exclusion process. There may have been relevant papers that were not initially included, based on the selection of search terms and databases (e.g., reports, unpublished papers), or studies that remain unpublished because of null findings. This review focuses on SAM use during young adulthood due to the high-risk nature

of this population. Thus, papers focused solely on adolescents younger than age 18 or adults older than age 30 were excluded. There is a growing body of work focused on unique circumstances of SAM use among adolescents,[93] and future work should continue to explore SAM use among other populations at risk. Additionally, the initial search may have missed papers that referenced general samples of adults more broadly if their abstracts did not mention the inclusion of young adults. Although all papers were independently reviewed by two authors to reduce bias, there may be instances when conceptualizations or terms identified as not fitting the current definition of SAM use were misinterpreted by both reviewers and thus excluded. Finally, this review focused on papers that included self-reported SAM use, survey research, and psychosocial-related variables, and did not review or report outcomes that were based on toxicology or medical reports; neurological, policy, or economic outcomes; or qualitative results. Such research may provide additional context for understanding SAM use, as well as its predictors and consequences, among young adults.

Conclusions

There continues to be an increasing research focus on SAM use, with new findings emerging quickly. To date, it is clear that SAM use is prevalent among young adults and is associated with perceived positive and negative consequences. However, much remains to be learned. In particular, the ways in which SAM use confers acute risk—above and beyond the risks associated with separate consumption of alcohol and marijuana—need to be identified. Psychosocial correlates identified so far include motives for SAM use and norms about use. Whether these additional constructs could be added to supplement existing alcohol- or marijuana-focused interventions, or whether new stand-alone SAM interventions are needed, remains to be seen. Increased understanding of the mechanisms by which SAM use leads to negative consequences is needed to design and test the most effective intervention strategies.

References

1. Schulenberg JE, Patrick ME, Johnston LD, O'Malley PM, Bachman JG, Miech RA. *Monitoring the Future National Survey Results on Drug Use, 1975-2020: Volume II, College Students and Adults Ages 19-60*. Institute for Social Research, The University of Michigan; 2021. http://monitoringthefuture.org/pubs/monographs/mtf-vol2_2020.pdf.
2. Duperrouzel JC, Granja K, Pacheco-Colón I, Gonzalez R. Adverse effects of cannabis use on neurocognitive functioning: A systematic review of meta-analytic studies. *J Dual Diagn*. 2020;16(1):43-57. https://doi.org/10.1080/15504263.2019.1626030.
3. Hingson R, Heeren T, Winter M, Wechsler H. Magnitude of alcohol-related mortality and morbidity among U.S. college students ages 18-24: Changes from 1998 to 2001. *Annu Rev Public Health*. 2005;26:259-279. https://doi.org/10.1146/annurev.publhealth.26.021304.144652.
4. Volkow ND, Baler RD, Compton WM, Weiss SRB. Adverse health effects of marijuana use. *N Engl J Med*. 2014;371(9):878-879. https://doi.org/10.1056/NEJMra1402309.
5. White A, Hingson R. The burden of alcohol use: Excessive alcohol consumption and related consequences among college students. *Alcohol Res*. 2013;35(2):201-218. https://arcr.niaaa.nih.gov/measuring-burden-alcohols-evolving-impact-individuals-families-and-society/burden-alcohol-use.
6. Subbaraman MS, Kerr W. Simultaneous versus concurrent use of alcohol and cannabis in the National Alcohol Survey. *Alcohol Clin Exp Res*. 2015;39(5):872-879. https://doi.org/10.1111/acer.12698.
7. Yurasek AM, Merrill JE, Metrik J, Miller MB, Fernandez AC, Borsari B. Marijuana use in the context of alcohol interventions for mandated college students. *J Subst Abuse Treat*. 2017;79:53-60. https://doi.org/10.1016/j.jsat.2017.05.015.
8. Agrawal A, Lynskey MT, Madden PAF, Bucholz KK, Heath AC. A latent class analysis of illicit drug abuse/dependence: Results from the National Epidemiological Survey on Alcohol and Related Conditions. *Addiction*. 2007;102(1):94-104. https://doi.org/10.1111/j.1360-0443.2006.01630.x.
9. Stinson FS, Ruan WJ, Pickering R, Grant BF. Cannabis use disorders in the USA: Prevalence, correlates and co-morbidity. *Psychol Med*. 2006;36(10):1447-1460. https://doi.org/10.1017/S0033291706008361.
10. McCabe SE, Arterberry BJ, Dickinson K, et al. Assessment of changes in alcohol and marijuana abstinence, co-use, and use disorders among US young adults from 2002 to 2018. *JAMA Pediatr*. 2021;175(1):64-72. https://doi.org/10.1001/jamapediatrics.2020.3352.
11. Guttmannova K, Lee CM, Kilmer JR, et al. Impacts of changing marijuana policies on alcohol use in the United States. *Alcohol Clin Exp Res*. 2016;40(1):33-46. https://doi.org/10.1111/acer.12942.
12. Subbaraman MS. Substitution and complementarity of alcohol and cannabis: A review of the literature. *Subst Use Misuse*. 2016;51(11):1399-1414. https:/doi.org/10.3109/10826084.2016.1170145.
13. Risso C, Boniface S, Subbaraman MS, Englund A. Does cannabis complement or substitute alcohol consumption? A systematic review of human and animal studies. *J Psychopharmacol*. 2020;34(9):938-954. https://doi.org/10.1177/0269881120919970.
14. Terry-McElrath YM, Patrick M. Simultaneous alcohol and marijuana use among young adult drinkers: Age-specific changes in prevalence from 1977-2016. *Alcohol Clin Exp Res*. 2018;42(11):2224-2233. https://doi.org/10.1111/acer.13879.
15. Patrick ME, Kloska DD, Terry-McElrath YM, Lee CM, O'Malley PM, Johnston LD. Patterns of simultaneous and concurrent alcohol and marijuana use among adolescents. *Am J Drug Alcohol Abuse*. 2018;44(4):441-451. https://doi.org/10.1080/00952990.2017.1402335.
16. Patrick M, Terry-McElrath YM, Lee C, Schulenberg J. Simultaneous alcohol and marijuana use among underage young adults in the United States. *Addict Behav*. 2019;88:77-81. https://doi.org/10.1016/j.addbeh.2018.08.015.
17. Sobell LC, Sobell MB. Timeline Follow-Back: A technique for assessing self-reported alcohol consumption. In: Litten RZ, Allen JP, eds. *Measuring Alcohol Consumption*. Totowa, NJ: Humana Press; 1992:41-72. http://doi.org/10.1007/978-1-4612-0357-5_3.
18. Shiffman S, Stone AA, Hufford MR. Ecological momentary assessment. *Annu Rev Public Health*. 2008;4:1-32. https://doi.org/10.1146/annurev.clinpsy.3.022806.091415.

19. Tricco AC, Lillie E, Zarin W, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and explanation. *Ann Intern Med.* 2018;169(7):467-473. https://doi.org/10.7326/M18-0850.
20. Terry-McElrath YM, O'Malley P, Johnston L. Alcohol and marijuana use patterns associated with unsafe driving among US high school seniors: High use frequency, concurrent use, and simultaneous use. *J Stud Alcohol Drugs.* 2014;75(3):378-389. https://doi.org/10.15288/jsad.2014.75.378.
21. Terry-McElrath YM, O'Malley PM, Johnston LD. Simultaneous alcohol and marijuana use among US high school seniors from 1976 to 2011: Trends, reasons, and situations. *Drug Alcohol Depend.* 2013;133(1):71-79. https://doi.org/10.1016/j.drugalcdep.2013.05.031.
22. Patrick M, Veliz P, Terry-McElrath YM. High-intensity and simultaneous alcohol and marijuana use among high school seniors in the United States. *Subst Abuse.* 2017;38(4):498-503. https://doi.org/10.1080/08897077.2017.1356421.
23. Midanik LT, Tam TW, Weisner C. Concurrent and simultaneous drug and alcohol use: Results of the 2000 National Alcohol Survey. *Drug Alcohol Depend.* 2007;90(1):72-80. https://doi.org/10.1016/j.drugalcdep.2007.02.024.
24. Jackson KM, Sokolovsky AW, Gunn RL, White HR. Consequences of alcohol and marijuana use among college students: Prevalence rates and attributions to substance-specific versus simultaneous use. *Psychol Addict Behav.* 2020;34(2):370-381. https://doi.org/10.1037/adb0000545.
25. Pakula B, Macdonald S, Stockwell T. Settings and functions related to simultaneous use of alcohol with marijuana or cocaine among clients in treatment for substance abuse. *Subst Use Misuse.* 2009;44(2):212-226. https:/doi.org/10.1080/10826080802347545.
26. Subbaraman MS, Kerr WC. Subgroup trends in alcohol and cannabis co-use and related harms during the rollout of recreational cannabis legalization in Washington state. *Int J Drug Policy.* 2020;75:102508. https://doi.org/10.1016/j.drugpo.2019.07.003.
27. de Oliveira LG, Alberghini DG, dos Santos B, de Andrade AG. Polydrug use among college students in Brazil: A nationwide survey. *Braz J Psychiatry.* 2013;35(3):221-230. https://doi.org/10.1590/1516-4446-2012-0775.
28. Thompson K, Holley M, Sturgess C, Leadbeater B. Co-use of alcohol and cannabis: Longitudinal associations with mental health outcomes in young adulthood. *Int J Environ Res Public Health.* 2021;18(7):3652. https://doi.org/10.3390/ijerph18073652.
29. Fairlie AM, Graupensperger S, Duckworth JC, Patrick ME, Lee CM. Unplanned versus planned simultaneous alcohol and marijuana use in relation to substance use and consequences: Results from a longitudinal daily study. *Psychol Addict Behav.* 2021;35(6):712-722. https://doi.org/10.1037/adb0000738.
30. Yeomans-Maldonado G, Patrick ME. The effect of perceived risk on the combined used of alcohol and marijuana: Results from daily surveys. *Addict Behav Rep.* 2015;2:33-36. https://doi.org/10.1016/j.abrep.2015.05.004.
31. Meisel MK, Treloar Padovano H, Miller MB, Clark MA, Barnett NP. Associations between social network characteristics and alcohol use alone or in combination with cannabis use in first-year college students. *Psychol Addict Behav.* 2021;35(6):650-658. https://doi.org/10.1037/adb0000704.
32. Lee CM, Patrick ME, Fleming CB, et al. A daily study comparing alcohol-related positive and negative consequences for days with only alcohol use versus days with simultaneous alcohol and marijuana use in a community sample of young adults. *Alcohol Clin Exp Res.* 2020;44(3):689-696. https://doi.org/10.1111/acer.14279.
33. Stevens AK, Aston ER, Gunn RL, et al. Does the combination matter? Examining the influence of alcohol and cannabis product combinations on simultaneous use and consequences in daily life. *Alcohol Clin Exp Res.* 2021;45(1):181-193. https://doi.org/10.1111/acer.14494.
34. Sukhawathanakul P, Thompson K, Brubacher J, Leadbeater B. Marijuana trajectories and associations with driving risk behaviors in Canadian youth. *Traffic Inj Prev.* 2019;20(5):472-477. https://doi.org/10.1080/15389588.2019.1622097.
35. Linden-Carmichael AN, Stamates A, Lau-Barraco C. Simultaneous use of alcohol and marijuana: Patterns and individual differences. *Subst Use Misuse.* 2019;54(13):2156-2166. https://doi.org/10.1080/10826084.2019.1638407.
36. Cummings C, Beard C, Habarth JM, Weaver C, Haas A. Is the sum greater than its parts? Variations in substance-related consequences by conjoint alcohol-marijuana use patterns. *J Psychoactive Drugs.* 2019;51(4):351-359. https://doi.org/10.1080/02791072.2019.1599473.
37. Collins RL, Bradizza CM, Vincent PC. Young-adult malt liquor drinkers: Predictors of alcohol problems and marijuana use. *Psychol Addict Behav.* 2007;21(2):138-146. https://doi.org/10.1037/0893-164X.21.2.138.
38. Looby A, Prince MA, Villarose-Hurlocker MC, et al. Young adult use, dual use, and simultaneous use of alcohol and marijuana: An examination of differences across use status on marijuana use context, rates, and consequences. *Psychol Addict Behav.* 2021;35(6):682-690. https://doi.org/10.1037/adb0000742.
39. Swan C, Ferro MA, Thompson K. Does how you use matter? The link between mode of use and cannabis-related risk. *Addict Behav.* 2021;112:106620. https://doi.org/10.1016/j.addbeh.2020.106620.
40. Arterberry BJ, Treloar H, McCarthy DM. Empirical profiles of alcohol and marijuana use, drugged driving, and risk perceptions. *J Stud Alcohol Drugs.* 2017;78(6):889-898. https://doi.org/10.15288/jsad.2017.78.889.
41. Cadigan JM, Dworkin ER, Ramirez JJ, Lee CM. Patterns of alcohol use and marijuana use among students at 2- and 4-year institutions. *J Am Coll Health.* 2019;67(4):383-390. https://doi.org/10.1080/07448481.2018.1484362.
42. Bailey AJ, Farmer EJ, Finn PR. Patterns of polysubstance use and simultaneous co-use in high risk young adults. *Drug Alcohol Depend.* 2019;205:107656. https://doi.org/10.1016/j.drugalcdep.2019.107656.
43. Linden-Carmichael AN, Allen HK. Profiles of alcohol and marijuana use among simultaneous alcohol and marijuana users: Individual differences in demographics and substance use. *J Drug Issues.* 2021;51(2):243-252. https://doi.org/10.1177/0022042620979617.
44. Stamates AL, Roberts R, Lau-Barraco C. Alcohol, cannabis, and tobacco polysubstance use: A latent profile analysis of age of onset. *Subst Abuse.* 2022;43(1):531-538. https://doi.org/10.1080/08897077.2021.1949777.
45. Linden-Carmichael AN, Van Doren N, Masters LD, Lanza ST. Simultaneous alcohol and marijuana use in daily life: Implications for level of use, subjective intoxication, and positive and negative consequences. *Psychol Addict Behav.* 2020;34(3):447-453. https://doi.org/10.1037/adb0000556.
46. Gunn RL, Norris AL, Sokolovsky A, Micalizzi L, Merrill JE, Barnett NP. Marijuana use is associated with alcohol use and consequences across the first 2 years of college. *Psychol Addict Behav.* 2018;32(8):885-894. https://doi.org/10.1037/adb0000416.
47. Metrik J, Gunn RL, Jackson KM, Sokolovsky AW, Borsari B. Daily patterns of marijuana and alcohol co-use among individuals with alcohol and cannabis use disorders. *Alcohol Clin Exp Res.* 2018;42(6):1096-1104. https://doi.org/10.1111/acer.13639.

48. Ito TA, Cordova KA, Skrzynski CJ, Bryan A. Complementarity in daily marijuana and alcohol among emerging adults. *Psychol Addict Behav.* 2021;35(6):723-736. https://doi.org/10.1037/adb0000771.
49. Sokolovsky AW, Gunn RL, Micalizzi L, White HR, Jackson KM. Alcohol and marijuana co-use: Consequences, subjective intoxication, and the operationalization of simultaneous use. *Drug Alcohol Depend.* 2020;212:107986. https://doi.org/10.1016/j.drugalcdep.2020.107986.
50. Gunn RL, Sokolovsky AW, Stevens AK, Metrik J, White H, Jackson K. Ordering in alcohol and cannabis co-use: Impact on daily consumption and consequences. *Drug Alcohol Depend.* 2021;218:108339. https://doi.org/10.1016/j.drugalcdep.2020.108339.
51. Linden-Carmichael AN, Allen H, Lanza S. The socio-environmental context of simultaneous alcohol and marijuana use among young adults: Examining day-level associations. *Drug Alcohol Rev.* 2021;40(4):647-657. https://doi.org/10.1111/dar.13213.
52. Lipperman-Kreda S, Paschall MJ, Saltz RF, Morrison CN. Places and social contexts associated with simultaneous use of alcohol, tobacco and marijuana among young adults. *Drug Alcohol Rev.* 2018;37(2):188-195. https://doi.org/10.1111/dar.12537.
53. D'Amico EJ, Rodriguez A, Tucker JS, et al. Early and late adolescent factors that predict co-use of cannabis with alcohol and tobacco in young adulthood. *Prev Sci.* 2020;21(4):530-544. https://doi.org/10.1007/s11121-020-01086-7.
54. Gunn RL, Sokolovsky A, Stevens AK, et al. Contextual influences on simultaneous alcohol and cannabis use in a predominately white sample of college students. *Psychol Addict Behav.* 2021;35(6):691-697. https://doi.org/10.1037/adb0000739.
55. Patrick ME, Fairlie AM, Lee CM. Motives for simultaneous alcohol and marijuana use among young adults. *Addict Behav.* 2018;76:363-369. https://doi.org/10.1016/j.addbeh.2017.08.027.
56. Conway FN, Sokolovsky A, White HR, Jackson KM. Simultaneous alcohol and marijuana use: A brief measure of motives. *J Stud Alcohol Drugs.* 2020;81(2):203-211. https://doi.org/10.15288/jsad.2020.81.203.
57. Arterberry BJ, Goldstick JE, Walton MA, Cunningham RM, Bonar EE. Alcohol and cannabis motives: Differences in daily motive endorsement on alcohol, cannabis, and alcohol/cannabis co-use days in a cannabis-using sample. *Addict Res Theory.* 2021;29(2):111-116. https://doi.org/10.1080/16066359.2020.1787390.
58. Patrick ME, Fleming CB, Fairlie AM, Lee CM. Cross-fading motives for simultaneous alcohol and marijuana use: Associations with young adults' use and consequences across days. *Drug Alcohol Depend.* 2020;213:108077. https://doi.org/10.1016/j.drugalcdep.2020.108077.
59. Patrick ME, Fairlie AM, Cadigan JM, Abdallah DA, Larimer ME, Lee CM. Daily motives for alcohol and marijuana use as predictors of simultaneous use among young adults. *J Stud Alcohol Drugs.* 2019;80(4):454-461. https://doi.org/10.15288/jsad.2019.80.454.
60. White HR, Kilmer JR, Fossos-Wong N, Hayes K, Sokolovsky AW. Simultaneous alcohol and marijuana use among college students: Patterns, correlates, norms, and consequences. *Alcohol Clin Exp Res.* 2019;43(7):1545-1555. https://doi.org/10.1111/acer.14072.
61. Patrick ME, Lee CM. Cross-faded: Young adults' language of being simultaneously drunk and high. *Cannabis.* 2018;1(2):60-65. https://doi.org/10.26828/cannabis.2018.02.006.
62. Ramirez JJ, Cadigan JM, Lee CM. Behavioral economic demand for alcohol among young adults who engage in simultaneous alcohol and marijuana use. *Subst Abuse.* 2020;41(2):203-270. https://doi.org/10.1080/08897077.2019.1671939.
63. Schuster RM, Mermelstein RJ, Hedeker D. Ecological momentary assessment of working memory under conditions of simultaneous marijuana and tobacco use. *Addiction.* 2016;111(8):1466-1476. https://doi.org/10.1111/add.13342.
64. Weiss NH, Bold KW, Sullivan TP, Armeli S, Tennen H. Testing bidirectional associations among emotion regulation strategies and substance use: A daily diary study. *Addiction.* 2017;112(4):695-704. https://doi.org/10.1111/add.13698.
65. Fleming CB, Duckworth JC, Rhew IC, et al. Young adult simultaneous alcohol and marijuana use: Between- and within-person associations with negative alcohol-related consequences, mental health, and general health across two-years. *Addict Behav.* 2021;123:107079. https://doi.org/10.1016/j.addbeh.2021.107079.
66. Baggio S, Sapin M, Khazaal Y, Studer J, Wolff H, Gmel G. Comorbidity of symptoms of alcohol and cannabis use disorders among a population-based sample of simultaneous users. Insight from a network perspective. *Int J Environ Res Public Health.* 2018;15(12):2893. https://doi.org/10.3390/ijerph15122893.
67. Brown WC, Testa M, Wang W. Alcohol and marijuana use in undergraduate males: Between- and within-person associations with interpersonal conflict. *Cannabis.* 2018;1(2):48-59. https://doi.org/10.26828/cannabis.2018.02.005.
68. Davis CN, Dash GF, Miller MB, Slutske WS. Past year high-intensity drinking moderates the association between simultaneous alcohol and marijuana use and blackout frequency among college students. *J Am Coll Health.* 2021:1-7. https://doi.org/10.1080/07448481.2021.1880415.
69. Mallett KA, Turrisi R, Trager BM, Sell N, Linden-Carmichael AN. An examination of consequences among college student drinkers on occasions involving alcohol-only, marijuana-only, or combined alcohol and marijuana use. *Psychol Addict Behav.* 2019;33(3):331-336. https://doi.org/10.1037/adb0000458.
70. Norman T, Peacock A, Bruno R, et al. Aggression in the Australian night time economy: A comparison of alcohol only versus alcohol and illicit drug consumption. *Drug Alcohol Rev.* 2019;38(7):744-749. https://doi.org/10.1111/dar.12998.
71. Graupensperger S, Fairlie AM, Vitiello MV, et al. Daily-level effects of alcohol, marijuana, and simultaneous use on young adults' perceived sleep health. *Sleep.* 2021;44(12):zsab187. https://doi.org/10.1093/sleep/zsab187.
72. Read JP, Colder CR, Livingston JA, Maguin E, Egerton G. Alcohol and cannabis co-use and social context as risk pathways to sexual assault. *Psychol Addict Behav.* 2021;35(6):659-670. https://doi.org/10.1037/adb0000737.
73. Stevens AK, Boyle HK, Sokolovsky AW, White HR, Jackson KM. Nuanced relations between simultaneous alcohol and cannabis use motives and negative consequences among college students: The role of multiple product use. *Exp Clin Psychopharmacol.* 2021:10.1037. https://doi.org/10.1037/pha0000454.
74. Egan KL, Cox MJ, Suerken CK, et al. More drugs, more problems? Simultaneous use of alcohol and marijuana at parties among youth and young adults. *Drug Alcohol Depend.* 2019;202:69-75. https://doi.org/10.1016/j.drugalcdep.2019.07.003.
75. Merrill JE, Boyle HK, Jackson KM, Carey KB. Event-level correlates of drinking events characterized by alcohol-induced blackouts. *Alcohol Clin Exp Res.* 2019;43(12):2599-2606. https://doi.org/10.1111/acer.14204.
76. Duckworth JC, Lee CM. Associations among simultaneous and co-occurring use of alcohol and marijuana, risky driving, and perceived risk. *Addict Behav.* 2019;96:39-42. https://doi.org/10.1016/j.addbeh.2019.03.019.
77. Thombs DL, O'Mara R, Dodd VJ, et al. Event-specific analyses of poly-drug abuse and concomitant risk behavior in a college bar district in Florida. *J Am Coll Health.* 2009;57(6):575-586. https://doi.org/10.3200/JACH.57.6.575-586.
78. Patrick ME, Graupensperger S, Dworkin ER, Duckworth JC, Abdallah DA, Lee CM. Intoxicated driving and riding with impaired drivers: Comparing days with alcohol, marijuana, and simultaneous use. *Drug Alcohol Depend.* 2021;225:108753. https://doi.org/10.1016/j.drugalcdep.2021.108753.

79. Hultgren BA, Waldron KA, Mallett KA, Turrisi R. Alcohol, marijuana, and nicotine use as predictors of impaired driving and riding with an impaired driver among college students who engage in polysubstance use. *Accid Anal Prev.* 2021;160:106341. https://doi.org/10.1016/j.aap.2021.106341.
80. Mallett KA, Bachrach RL, Turrisi R. Are all negative consequences truly negative? Assessing variations among college students' perceptions of alcohol related consequences. *Addict Behav.* 2008;33(10):1375-1381. https://doi.org/10.1016/j.addbeh.2008.06.014.
81. Patrick ME, Maggs JL. College students' evaluations of alcohol consequences as positive and negative. *Addict Behav.* 2011;36(12):1148-1153. https://doi.org/10.1016/j.addbeh.2011.07.011.
82. Gmel G, Kuendig H, Rehm J, Schreyer N, Daeppen JB. Alcohol and cannabis use as risk factors for injury – a case-crossover analysis in a Swiss hospital emergency department. *BMC Public Health.* 2009;9:40. https://doi.org/10.1186/1471-2458-9-40.
83. Lewis MA, Neighbors C. Social norms approaches using descriptive drinking norms education: A review of the research on personalized normative feedback. *J Am Coll Health.* 2006;54(4):213-218. https://doi.org/10.3200/JACH.54.4.213-218.
84. Volkow N. Establishing 5mg of THC as the standard unit for research. Nora's Blog, National Institute on Drug Abuse website. 2021. https://www.drugabuse.gov/about-nida/noras-blog/2021/05/establishing-5mg-thc-standard-unit-research.
85. National Institute on Drug Abuse; National Heart, Lung, and Blood Institute; National Institute of Mental Health; National Cancer Institute. Notice of information: Establishment of a standard THC unit to be used in research. Notice No.: NOT-DA-21-049. NIH Grants, 2021. https://grants.nih.gov/grants/guide/notice-files/NOT-DA-21-049.html.
86. Cronce JM, Toomey TL, Lenk K, Nelson TF, Kilmer JR, Larimer ME. NIAAA's college alcohol intervention matrix: CollegeAIM. *Alcohol Res.* 2018;39(1):43-47.
87. White HR, Jiao Y, Ray AR, et al. Are there secondary effects on marijuana use from brief alcohol interventions for college students? *J Stud Alcohol Drugs.* 2015;76(3):367-377. https://doi.org/10.15288/jsad.2015.76.367.
88. Halladay J, Scherer J, MacKillop J, et al. Brief interventions for cannabis use in emerging adults: A systematic review, meta-analysis, and evidence map. *Drug Alcohol Depend.* 2019;204:107565. https://doi.org/10.1016/j.drugalcdep.2019.107565.
89. Lee CM, Kilmer JR, Neighbors C, et al. Indicated prevention for college student marijuana use: A randomized controlled trial. *J Consult Clin Psychol.* 2013;81(4):702-709. https://doi.org/10.1037/a0033285.
90. Yurasek AM, Aston ER, Metrik J. Co-use of alcohol and cannabis: A review. *Curr Addict Rep.* 2017;4(2):184-193. https://doi.org/10.1007/s40429-017-0149-8.
91. Stein MD, Caviness CM, Morse EF, et al. A developmental-based motivational intervention to reduce alcohol and marijuana use among non-treatment-seeking young adults: A randomized controlled trial. *Addiction.* 2018;113(3):440-453. https://doi.org/10.1111/add.14026.
92. Woolard R, Baird J, Longabaugh R, et al. Project Reduce: Reducing alcohol and marijuana misuse: Effects of a brief intervention in the emergency department. *Addict Behav.* 2013;38(3):1732-1739. https://doi.org/10.1016/j.addbeh.2012.09.006.
93. Lipperman-Kreda S, Gruenewald PJ, Grube JW, Bersamin M. Adolescents, alcohol, and marijuana: Context characteristics and problems associated with simultaneous use. *Drug Alcohol Depend.* 2017;179:55-60. https://doi.org/10.1016/j.drugalcdep.2017.06.023.
94. Roche DJ, Bujarski S, Green R, et al. Alcohol, tobacco, and marijuana consumption is associated with increased odds of same-day substance co-use and tri-use. *Drug Alcohol Depend.* 2019;200:40-49. https://doi.org/10.1016/j.drugalcdep.2019.02.035.
95. Barrett SP, Darredeau C, Pihl RO. Patterns of simultaneous polysubstance use in drug using university students. *Hum Psychopharmacol.* 2006;21(4):255-263. https://doi.org/10.1002/hup.766.
96. Licht CL, Christofferson M, Okhold M, et al. Simultaneous polysubstance use among Danish 3,4-methylenedioxymethamphetamine and hallucinogen users: Combination patterns and proposed biological bases. *Human Psychopharmacol.* 2012;27(4):352-363. https://doi.org/10.1002/hup.2234.
97. Olthuis JV, Darredeau C, Barrett SP. Substance use initiation: The role of simultaneous polysubstance use. *Drug Alcohol Rev.* 2013;32(1):67-71. https://doi.org/10.1111/j.1465-3362.2012.00470.x.
98. Østergaard J, Østergaard SV, Fletcher A. Preferences for simultaneous polydrug use: A comparative study of young adults in England and Denmark. *Contemp Drug Probl.* 2016;43(4):350-368. https://doi.org/10.1177/0091450916661372.
99. Wade NE, Thomas AM, Gruber SA, Tapert SF, Filbey FM, Lisdahl KM. Binge and cannabis co-use episodes in relation to white matter integrity in emerging adults. *Cannabis Cannabinoid Res.* 2020;5(1):62-72. https://doi.org/10.1089/can.2018.0062.
100. Coughlin LN, Bonar EE, Bohnert ASB, et al. Patterns of same-day alcohol and cannabis use in adolescents and young adults with risky alcohol use. *Addict Res Theory.* 2021. https://doi.org/10.1080/16066359.2021.1936511.
101. Linden-Carmichael AN, Hochgraf AK, Cloutier RM, Stull SW, Lanza ST. Associations between simultaneous alcohol and cannabis use and next-day negative affect among young adults: The role of sex and trait anxiety. *Addict Behav.* 2021;123:107082. https://doi.org/10.1016/j.addbeh.2021.107082.
102. Daros AR, Pereira BJ, Khan D, Ruocco AC, Quilty LC, Wardell JD. Daily associations between cannabis use and alcohol use in young adults: The moderating role of self-report and behavioral measures of impulsivity. *Addict Res Theory.* 2021. https://doi.org/10.1080/16066359.2021.1939314.
103. Lee CM, Cadigan JM, Patrick ME. Differences in reporting of perceived acute effects of alcohol use, marijuana use, and simultaneous alcohol and marijuana use. *Drug Alcohol Depend.* 2017;180:391-394. https://doi.org/10.1016/j.drugalcdep.2017.08.029.

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Subbaraman & Kerr, 2015	6	Cross-sectional	National sample from National Alcohol Survey (2005 and 2010)	Age group 18–29	8,626	SAM use: Unspecified overlap	✓			✓
Terry-McElrath & Patrick, 2018	14	Longitudinal; Panel	Nationally representative sample of 12th-grade students from Monitoring the Future	NR	11,789	SAM use: Overlapping effects	✓			
Patrick et al., 2018	15	Cross-sectional	Nationally representative sample of 12th-grade students from Monitoring the Future survey; sample limited to cases from 1976 to 2016 that reported past-year alcohol and marijuana use	NR	84,805	SAM use: Overlapping effects	✓	✓		
Patrick et al., 2019	16	Longitudinal; Panel	Nationally representative sample of 12th-grade students from Monitoring the Future who participated in longitudinal follow-up at modal ages 19 or 20 from 2007 to 2016	NR	1,719	SAM use: Overlapping effects	✓	✓		
Terry-McElrath, O'Malley, & Johnston, 2014	20	Cross-sectional	Nationally representative sample of 12th-grade students from Monitoring the Future	NR	72,053	SAM use: Overlapping effects	✓	✓		✓

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Terry-McElrath et al., 2013	21	Cross-sectional	Nationally representative sample of 12th-grade students from Monitoring the Future	NR	34,850	SAM use: Overlapping effects	✓	✓	✓	
Patrick et al., 2017	22	Cross-sectional	Nationally representative sample of 12th-grade students from Monitoring the Future	NR	24,203	SAM use: Overlapping effects	✓	✓		
Midanik et al., 2007	23	Cross-sectional	National sample from National Alcohol Survey (1999–2001)	Age group 18–29	4,630	SAM use: Unspecified overlap	✓	✓	✓	✓
Jackson et al., 2020	24	Cross-sectional	College students who reported past-year alcohol and marijuana use	Age group 18–24	1,390	SAM use: Overlapping effects	✓	✓		✓
Pakula, Macdonald, & Stockwell, 2009	25	Cross-sectional	Clients from treatment programs in Canada reporting past-year marijuana or cocaine use	Age group 18–29	499	SAM use: Unspecified overlap	✓		✓	
Subbaraman & Kerr, 2020	26	Cross-sectional	Sample includes six representative surveys of adults in Washington state between January 2014 and October 2016	Age group 18–29	5,492	SAM use: Unspecified overlap	✓			
de Oliveira et al., 2013	27	Cross-sectional	Nationwide sample of Brazilian college students	Age group 18–24	12,544	SAM use: Unspecified overlap	✓			

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Thompson et al., 2021	28	Longitudinal; Panel	Community sample of youth in 10-year longitudinal study with biennial surveys; data from time points 5 and 6	Time 5 Ages 20–26 Time 6 Ages 22–29	Time 5 464 Time 6 478	SAM use: Time frame specified	✓	✓	✓	✓
Fairlie et al., 2021	29	Longitudinal; Daily/EMA	Community sample who reported SAM use at least once in past 2 weeks and alcohol use at least three times in past month	Age group 18–25	Baseline 409 Daily SAM 322 Daily unplanned SAM 308	SAM use: Overlapping effects	✓			✓
Yeomans-Maldnado & Patrick, 2015	30	Longitudinal; Daily/EMA	12th-grade students in the Midwest who participated in a baseline survey and completed at least one follow-up wave and daily survey	Follow-up X_{age} = 18.3	89	SAM use: Overlapping effects	✓		✓	
Meisel et al., 2021	31	Longitudinal; Panel	Incoming first-year college students	Age group 17–23	1,294	SAM use: Time frame specified		✓	✓	
Lee et al., 2020	32	Longitudinal; Daily/EMA	Community sample who reported SAM use at least once in past 2 weeks and alcohol use at least three times in past month	Age group 18–25	391	SAM use: Overlapping effects		✓		✓
Stevens et al., 2021	33	Longitudinal; Daily/EMA	College students who reported past-year alcohol and marijuana use and past-month SAM use	Age group 18–24	274	SAM use: Overlapping effects		✓		✓

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Sukhawathanakul et al., 2019	34	Longitudinal; Panel	Youth who participated in the biennial Victoria Healthy Youth Survey from 2003 to 2013	Age group 22–28	640	SAM use: Time frame specified		✓		✓
Linden-Carmichael, Stamates, & Lau-Barraco, 2019	35	Cross-sectional	National sample who reported alcohol use in the past month	Age group 18–25	1,017	SAM use: Time frame specified		✓	✓	✓
Cummings et al., 2019	36	Cross-sectional	First-year college students who reported any past 3-month substance use	X_{age} = 18.1	610	SAM use: Unspecified overlap		✓		✓
Collins, Bradizza, & Vincent, 2007	37	Cross-sectional	Community and college sample who reported drinking at least one 40 oz container of malt liquor a week	Age group 18–35	639	SAM use: Unspecified overlap		✓		
Looby et al., 2021	38	Cross-sectional	College students from seven universities across six states	X_{age} = 19.9	4,764	SAM use: Unspecified overlap		✓	✓	✓
Swan, Ferro, & Thompson, 2021	39	Cross-sectional	College students from a university in Canada, restricted to those who used cannabis in the last 6 months	Age group 17–26	368	SAM use: Time frame specified		✓		
Arterberry, Treloar, & McCarthy, 2017	40	Cross-sectional	College students in an introductory psychology class at a large, public university	X_{age} = 19.0	897	SAM use: Unspecified overlap		✓		

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Cadigan et al., 2019	41	Cross-sectional	Community sample who drank at least once in the past year and are currently enrolled in a 2- or 4-year institution	Age group 18–23	526	SAM use: Unspecified overlap		✓		
Bailey, Farmer, & Finn, 2019	42	Cross-sectional	Sample recruited for overrepresentation of externalizing problems	Age group 18–30	2,098	SAM use: Unspecified overlap		✓		
Linden-Carmichael & Allen, 2021	43	Cross-sectional	Young adults who reported past-month HED and SAM use	Age group 18–25	522	SAM use: Overlapping effects		✓		
Stamates, Roberts, & Lau-Barraco, 2021	44	Cross-sectional	Community sample who reported past-year alcohol, cannabis, and tobacco use	Age group 18–25	510	SAM use: Time frame specified	✓			
Linden-Carmichael et al., 2020	45	Longitudinal; Daily/EMA	Sample recruited near large, public university who reported past-month SAM use and HED in past 2 weeks	Age group 18–25	154	SAM use: Overlapping effects		✓		✓
Gunn et al., 2018	46	Longitudinal; TLFB	Incoming first-year college students in 2-year longitudinal study who reported at least one episode of alcohol and marijuana use during data collection	Baseline X_{age} = 18.4	488	Same-day co-use		✓		
Metrik et al., 2018	47	Longitudinal; TLFB	Veterans who used alcohol and marijuana on at least 1 day in the 180-day TLFB assessment period	X_{age} = 30.0	127	Same-day co-use		✓		

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Ito et al., 2021	48	Longitudinal; TLFB	College students in Colorado during the time period when recreational marijuana was decriminalized then legalized	X_{age} = 18.4	375	Same-day co-use		✓		
Sokolovsky et al., 2020	49	Longitudinal; Daily/EMA	College students who reported past-year alcohol and marijuana use and past-month SAM use	X_{age} = 19.8	341	SAM use: Time frame specified		✓		✓
Gunn et al., 2021	50	Longitudinal; Daily/EMA	College students who reported past-year alcohol and marijuana use and past-month SAM use	Age group 18–24	258	Same-day co-use		✓		✓
Linden-Carmichael, Allen, & Lanza, 2021	51	Longitudinal; Daily/EMA	Sample recruited near large, public university who reported past-month SAM use and HED in past 2 weeks	Age group 18–25	148	SAM use: Overlapping effects			✓	
Lipperman-Kreda et al., 2018	52	Cross-sectional	Youth who participated in a randomized community trial in California	Age group 18–30	1,538	SAM use: Unspecified overlap			✓	
D'Amico et al., 2020	53	Longitudinal; Panel	Youth who originally participated in a substance use prevention program in middle school	Follow-up X_{age} = 20.7	2,429	SAM use: Unspecified overlap			✓	
Gunn et al., 2021	54	Longitudinal; Daily/EMA	College students who reported past-year alcohol and marijuana use and past-month SAM use	Age group 18–24	313	SAM use: Time frame specified			✓	

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Patrick, Fairlie, & Lee, 2018	55	Cross-sectional	Community sample who, at recruitment, reported drinking at least once in the past year	X_{age} = 21.4	286	SAM use: Overlapping effects			✓	✓
Conway et al., 2020	56	Longitudinal; Panel	College students who reported past-year alcohol and marijuana use and SAM use	Age group 18–24	Baseline 1,014 Follow-up 904	SAM use: Overlapping effects			✓	✓
Arterberry et al., 2021	57	Longitudinal; Daily/EMA	Emergency department attendees who reported illicit drug use or prescription drug misuse in past 4 weeks	Age group 18–25	97	Same-day co-use			✓	
Patrick et al., 2020	58	Longitudinal; Daily/EMA	Community sample who reported SAM use at least once in past 2 weeks and alcohol use at least three times in past month	Age group 18–25	281	SAM use: Overlapping effects			✓	✓
Patrick et al., 2019	59	Longitudinal; Daily/EMA	Community sample who reported SAM use at least once in past 2 weeks and alcohol use at least three times in past month	Age group 18–25	399	SAM use: Overlapping effects			✓	
White et al., 2019	60	Cross-sectional	College students who reported past-year alcohol and marijuana use	Age group 18–24	1,389	SAM use: Overlapping effects			✓	✓

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Patrick & Lee, 2018	61	Cross-sectional	Community sample from Washington; screening survey for longitudinal study on social role transitions and alcohol use	Age group 18–23	807	SAM use: Unspecified overlap			✓	
Ramirez, Cadigan, & Lee, 2020	62	Cross-sectional	Community sample who, at recruitment, reported drinking at least once in past year	X_{age} = 21.9	480	SAM use: Overlapping effects			✓	
Schuster, Mermelstein, & Hedeker, 2016	63	Longitudinal; Daily/EMA	Youth who participated in study on smoking and reported at least one episode of marijuana, tobacco, or alcohol use during 5-year follow-up EMA	Follow-up X_{age} = 21.3	287	SAM use: Unspecified overlap			✓	
Weiss et al., 2017	64	Longitudinal; Daily/EMA	Undergraduate psychology students who reported alcohol use at least twice in the past month	X_{age} = 19.2	1,640	SAM use: Unspecified overlap			✓	
Fleming et al., 2021	65	Longitudinal; Panel	Community sample who, at recruitment, reported drinking at least once in the past year	Age group 18–23	773	SAM use: Overlapping effects			✓	✓
Baggio et al., 2018	66	Longitudinal; Panel	Swiss men recruited from national military recruitment centers who reported SAM use in the past year	Baseline X_{age} = 20.0 Follow-up X_{age} = 21.3	Baseline 1,559 Follow-up 991	Same-day co-use			✓	

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Brown, Testa, & Wang, 2018	67	Longitudinal; Daily/EMA	First-year college males from large public university	Age group 18–19	427	SAM use: Time frame specified				✓
Davis et al., 2021	68	Cross-sectional	College student sample; for interactive effects, subset of students who consumed alcohol in past year	X_{age} = 18.4	Prevalence 1,234 Interactive effects 997	SAM use: Unspecified overlap				✓
Mallett et al., 2019	69	Longitudinal; Daily/EMA	Third-year college students from a large, public university who were part of a longitudinal study and reported alcohol and other drug use in the past year	X_{age} = 20.1	451	Same-day co-use				✓
Norman et al., 2019	70	Cross-sectional	Individuals in Australia who went to bars or clubs	Age group 20–27	5,078	SAM use: Unspecified overlap				✓
Graupensperger et al., 2021	71	Longitudinal; Daily/EMA	Community sample who reported SAM use at least once in past 2 weeks and alcohol use at least three times in past month	Age group 18–25	409	SAM use: Overlapping effects				✓
Read et al., 2021	72	Longitudinal; Daily/EMA	Females who were part of a long-term longitudinal study on adolescent substance risk	Age group 21–24	174	Same-day co-use				✓
Stevens et al., 2021	73	Longitudinal; Daily/EMA	College students who reported past-year use of alcohol and marijuana	Age group 18–24	281	SAM use: Overlapping effects				✓

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Egan et al., 2019	74	Cross-sectional	Youth who participated in a randomized community trial	Age group 15–20	834	SAM use: Unspecified overlap				✓
Merrill et al., 2019	75	Longitudinal; Daily/EMA	College students who reported weekly HED or experiencing at least one negative alcohol-related consequence in past 2 weeks	Age group 18–20	96	SAM use: Unspecified overlap				✓
Duckworth & Lee, 2019	76	Cross-sectional	Community sample who, at recruitment, reported drinking at least once in the past year; data from Month 18	X_{age} = 22.2	511	SAM use: Overlapping effects				✓
Thombs et al., 2009	77	Cross-sectional	Patrons exiting bars in college bar district	Median age = 21	469	SAM use: Unspecified overlap				✓
Patrick et al., 2021	78	Longitudinal; Daily/EMA	Community sample who reported SAM use at least once in past 2 weeks and alcohol use at least three times in past month	Age group 18–25	408	SAM use: Overlapping effects				✓
Hultgren et al., 2021	79	Longitudinal; TLFB	College students who reported past-year use of alcohol and another substance (e.g., marijuana, nicotine)	X_{age} = 20.1	367	Same-day co-use				✓
Roche et al., 2019	94†	Longitudinal; TLFB	Non–treatment-seeking regular drinkers in Los Angeles area	X_{age} = 29.0	179	Same-day co-use				

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Barrett, Darredeau, & Pihl, 2006	95†	Cross-sectional	College students who reported use of at least two substances in their lifetime	X_{age} = 21.7	149	SAM use: Unspecified overlap				
Licht et al., 2012	96†	Cross-sectional	Danish adults who reported lifetime history of at least 15 illicit drug experiences (excluding marijuana) and use of MDMA or hallucinogens at least once in the past year	Age group 18–35	59	SAM use: Unspecified overlap				
Olthuis, Darredeau, & Barrett, 2013	97†	Cross-sectional	Community sample from Canada who reported lifetime cannabis use	X_{age} = 26.8	226	SAM use: Unspecified overlap				
Østergaard, Østergaard, & Fletcher, 2016	98†	Cross-sectional	Bar or club goers in Denmark and England	Age group 18–35	1,019	SAM use: Unspecified overlap				
Wade et al., 2020	99†	Cross-sectional	Community sample in Wisconsin	Age group 16–26	75	Same-day co-use				
Coughlin et al., 2021	100†	Longitudinal; TLFB	Community sample who reported risky alcohol use in past 3 months and at least 1 day of alcohol use and 1 day of cannabis use in past 30 days	Age group 16–24	468	Same-day co-use				
Linden-Carmichael et al., 2021	101†	Longitudinal; Daily/EMA	Community sample who reported past-month SAM use and past 2-week HED	Age group 18–25	154	SAM use: Overlapping effects				

Appendix 1. Sample Descriptions, Categorization of Simultaneous Alcohol and Marijuana (SAM) Use, and Areas of Focus Related to Narrative Review (Continued)

Citation Author & Year	Citation Number	Study Design	Population	Age (range or mean)	Sample Size	Categorization of SAM*	Inclusion in Narrative Results			
							Prevalence	Patterns	Correlates	Consequences
Daros et al., 2021	102†	Longitudinal; TLFB	Community sample of regular cannabis users (at least once per month for 6+ months) in Canada	Age group 19–26	153	Same-day co-use				
Lee, Cadigan, & Patrick, 2017	103†	Cross-sectional	Community sample who, at recruitment, reported drinking at least once in the past year	X_{age} = 21.4	315	SAM use: Overlapping effects				

***Categorization of SAM use.** SAM use: Overlapping effects = At the same time or together so that their effects overlapped; SAM use: Time frame specified = On the same day within a specified time period (e.g., within 3 hours of each other); SAM use: Unspecified overlap = At the same time or together without specifying that their effects overlapped or at the same event or occasion without specifying overlapping effects of use within a specified time period (e.g., at the last party attended, during the current night out); Same-day co-use = On the same day without specifying that they be used together or within a specified time period.

†Ten papers were identified in the search process and included through data extraction; however, the focus of each paper was outside the specific topics of the current review, or results related to SAM were mostly descriptive and thus not presented in the narrative synthesis.

Note. EMA, ecological momentary assessment; HED, heavy episodic drinking; MDMA ("ecstasy"), 3,4-methylenedioxy-N-methylamphetamine; NR, Not reported; SAM, simultaneous alcohol and marijuana; TLFB, timeline follow-back; X_{age}, mean age.

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2022;42(1):06 | https://doi.org/10.35946/arcr.v42.1.06 Published: 17 March 2022

Relationships of Cannabis Policy Liberalization With Alcohol Use and Co-Use With Cannabis: A Narrative Review

Rosalie Liccardo Pacula,[1] Rosanna Smart,[2] Marlene C. Lira,[3] Seema Choksy Pessar,[1] Jason G. Blanchette,[4] and Timothy S. Naimi[5]

[1]University of Southern California, Los Angeles, California, USA
[2]RAND Corporation, Santa Monica, California, USA
[3]Boston Medical Center, Boston, Massachusetts, USA
[4]Boston University School of Public Health, Boston, Massachusetts, USA
[5]Canadian Institute for Substance Use Research, Victoria, British Columbia, Canada

Correspondence
Address correspondence concerning this article to Rosalie Liccardo Pacula, Ph.D., Sol Price School of Public Policy, Schaeffer Center for Health Policy & Economics, University of Southern California, 635 Downey Way, VPD 514J, Los Angeles, CA 90089-3333. Email: Rmp_302@healthpolicy.usc.edu

Acknowledgments
This work was supported by National Institute on Alcohol Abuse and Alcoholism grant R01AA026268. The authors would like to acknowledge and thank Anita Szafran for her outstanding assistance with the online search.

Disclosures
The authors declare no competing financial or nonfinancial interests.

Publisher's Note
Opinions expressed in contributed articles do not necessarily reflect the views of the National Institute on Alcohol Abuse and Alcoholism, 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.

PURPOSE: The liberalization of cannabis policies has the potential to affect the use of other substances and the harms from using them, particularly alcohol. Although a previous review of this literature found conflicting results regarding the relationship between cannabis policy and alcohol-related outcomes, cannabis policies have continued to evolve rapidly in the years since that review.
SEARCH METHODS: The authors conducted a narrative review of studies published between January 1, 2015, and December 31, 2020, that assessed the effects of cannabis policies on the use of alcohol in the United States or Canada.
SEARCH RESULTS: The initial search identified 3,446 unique monographs. Of these, 23 met all inclusion criteria and were included in the review, and five captured simultaneous or concurrent use of alcohol and cannabis.
DISCUSSION AND CONCLUSIONS: Associations between cannabis policy liberalization and alcohol use, alcohol-related outcomes, and the co-use of alcohol and cannabis were inconclusive, with studies finding positive associations, no associations, and negative associations. Although several studies found that cannabis policy liberalization was associated with decreases in alcohol use measures, these same studies showed no impact of the cannabis policy on cannabis use itself. The lack of a consistent association was robust to subject age, outcome measure (e.g., use, medical utilization, driving), and type of cannabis policy; however, this may be due to the small number of studies for each type of outcome. This paper discusses several notable limitations of the evidence base and offers suggestions for improving consistency and comparability of research going forward, including a stronger classification of cannabis policy, inclusion of measures of the alcohol policy environment, verification of the impact of cannabis policy on cannabis use, and consideration of mediation effects.

KEYWORDS: cannabis; marijuana; policy; alcohol; outcomes; co-use; public health

For the past 25 years, a growing number of U.S. states have been progressively legalizing cannabis markets, first through the early adoption of medical cannabis laws, which enabled the purchase and possession of cannabis for specific medicinal purposes, and then more recently through laws regarding adult (i.e., "recreational") use of cannabis. As of May 2021, more than 70% of U.S. states (n = 36) allowed for medical markets of cannabis and 18 states and the District of Columbia had passed laws allowing for the recreational use of cannabis by adults,[1] despite federal prohibition. A key public health concern throughout much of this state policy innovation over the past 2 decades is the impact these cannabis liberalization laws might have on alcohol use and alcohol-related harm.[2,3] Although the harms caused by persistent use of cannabis, particularly high-potency cannabis, are still under scientific investigation,[4,5] the known harms associated with alcohol use are well established.[6]

Some have argued that cannabis use may be a substitute for alcohol consumption and, therefore, that liberalizing cannabis policies should reduce excessive drinking and alcohol-related harms. However, during the past 2 decades, there has been a consistent upward trend in alcohol consumption, as measured by per capita consumption[7,8] and self-reported annual and 30-day alcohol use prevalence rates.[9] This has occurred during the same period as a liberalization of cannabis policies. Significant research shows that cannabis use is a risk factor for underage drinking, excessive drinking, and crash fatalities involving alcohol,[10] supporting the notion that the liberalizing of cannabis policies may be contributing to the rise in alcohol use. Cannabis use among people who report drinking in the past month or past year (i.e., concurrent use among drinkers) remains fairly low overall, with approximately 15% of drinkers reporting cannabis use in the same month or past year.[11-13] However, concurrent use of alcohol among cannabis users is quite a bit higher, with more than 75% of cannabis users reporting alcohol use within the same 30-day period.[14-17] As more liberal cannabis laws generate more adult cannabis users,[18] there is concern that such laws may be resulting in more concurrent use of cannabis and alcohol as well.

Even more disconcerting is the evidence that two-thirds of those who both drink alcohol and use cannabis consume the substances simultaneously[11-13]—that is, during the same occasion. Recent evidence from the Monitoring the Future survey shows that the prevalence of simultaneous use of alcohol and marijuana (SAM) among young adults who drink (ages 19 to 22) is as high as 30%, and that of slightly older young adults (ages 23 to 30) is between 20% and 25%.[19] Moreover, between 1992 and 2016, there has been a consistent and significant increase in the prevalence of SAM among people ages 21 to 26 who drink alcohol, although the prevalence of SAM among people who use cannabis has been relatively stable.[19]

This growing evidence of simultaneous use of these two substances among people who drink alcohol has some public health advocates concerned that cannabis liberalization policies may be leading to more, not less, alcohol use and even more concurrent or simultaneous use. Compared with alcohol use alone, studies have shown two to three times increased odds of adverse social consequences (e.g., legal issues, relationship and financial problems) associated with co-use of alcohol and cannabis,[12,13,20] and simultaneous use is known to lead to greater cognitive, perceptual, and motor function impairment than using either alcohol or cannabis alone.[4,21-23]

The relationship between the consumption of cannabis and alcohol in a population will be influenced by a multitude of individual and environmental factors, including socio-demographics, cultural norms, perceived harm, and the general availability of both substances. Policy plays a role in influencing perceptions and norms by specifying exposure as well as access and price, which influence general availability.[24,25] Therefore, when big changes in policies targeting one substance occur, such as those recently directed toward cannabis, they provide a useful opportunity for identifying the true relationship between the demand for the targeted substance and any related substance, in this case alcohol. However, as Figure 1 shows, the opportunity to identify the nature of the relationship between cannabis and alcohol using this sort of large change in policy toward cannabis requires two things: (1) clear evidence that the policy in question changes the use of cannabis, the substance it actually is targeting, and (2) accounting for variation in any other alcohol policy that also might be changing and influencing consumption of alcohol (and possibly cannabis) at the same time.

Several studies published in economics journals suggest that evidence from state policies supports the conclusion that alcohol and cannabis are economic substitutes.[26-28] Yet, findings in the broader public health and sociology literature have been unable to draw such a firm conclusion.[21,29-31] The difference may be due to how economists strictly define substitutes and complements, using information gleaned from cross-price effects and their impacts on the budget constraint, or it may be due to methods relied on for causal inference.

This study updates and extends the 2016 review by Guttmannova et al., which summarized the findings regarding substitution and complementary use of alcohol and cannabis from published literature through 2015.[31] However, unlike the prior review, this article applies specific methodological standards associated with supporting causal inference[32,33] in screening the studies reviewed, with the goal of generating a more consistent interpretation of the literature evaluating the impact of cannabis policy on alcohol use and co-use with cannabis. This review discusses differences in effects identified across age groups, measures of cannabis policy (medical marijuana laws or recreational marijuana laws), and polydrug use (simultaneous and/or concurrent use).

By focusing on studies that generate findings from policy variation, this paper excludes studies such as those conducted by Park et al.[34] and O'Hara et al.,[35] which also examined the relationship between alcohol and cannabis use, but without considering the role policies play by influencing the relative

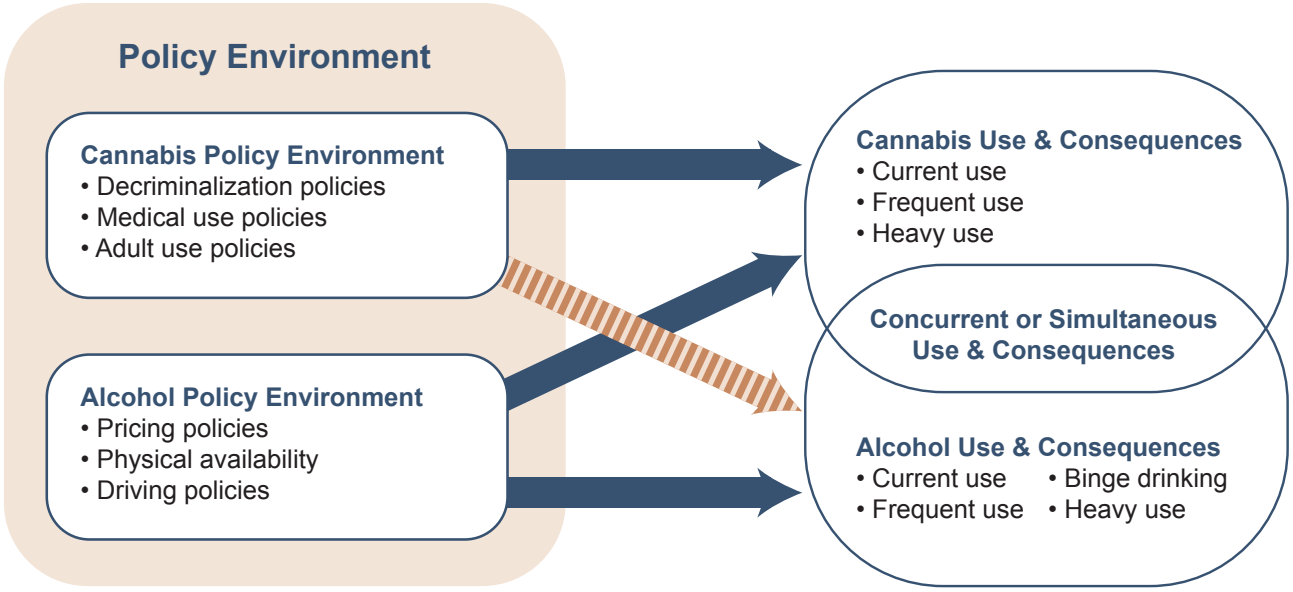

Figure 1. Relationship between cannabis and alcohol policy and use. Arrows represent existing relationships, with the striped orange arrow representing the relationship addressed in this review (i.e., the effects of cannabis policies on alcohol use as well as simultaneous cannabis and alcohol use and their consequences).

access and price of each substance. Economists rely on changes in patterns of use associated with exogenous shifts in prices, particularly the full price of related goods, for identification of economic substitutes or complements. Legal policy changes influence the monetary and legal cost of accessing a substance, and hence they are considered components of the full price of a substance.[24] However, as shown in this review, many studies, including those within the economics literature, have relied on a relatively weak measure of state alcohol policy, the beer-specific excise tax. Prior work has shown that over the past 20 years, the beer-specific excise tax accounts for a small percentage of taxes and is a poor indicator of alcohol taxes compared to measures incorporating multiple tax and beverage types.[36,37] Exclusion of the many additional dimensions of alcohol policy measures that influence the alcohol policy environment and the full price of alcohol may lead to an omitted variable bias when examining the impact of changes in cannabis policy. Thus, a key contribution of this literature review is its consideration of the extent to which studies have appropriately considered the true availability of alcohol while interpreting findings related to cannabis policy.

Material and Methods

Search Strategy

The authors followed many of the PRISMA 2020 Guidelines for conducting and reporting the findings from this literature review.[38] An online literature search was conducted for articles published between January 1, 2015, and December 31, 2020, using the following databases: EBSCO (which includes Academic Search Complete, American Psychological Association (APA) PsycInfo, Criminal Justice Abstracts, EconLit, Index to Legal Periodicals & Books, National Criminal Justice Reference Service Abstracts, and Social Sciences Abstracts), APA PsycArticles, PubMed, Scopus, Sociological Abstracts, and Web of Science. Additional search limiters were imposed related to language (English only) and study setting (United States and Canada only); and nonhuman studies, conference abstracts, and dissertations were explicitly excluded. The search terms used closely follow those of Guttmannova et al.,[31] with two important differences. First, some additional search terms were included to capture more inclusively cannabis and alcohol use behaviors (e.g., the terms "cannabis," "beer," "wine," and "spirit" had all been excluded from the Guttmannova study[31] but were included in this study). Second, this study excluded the requirement that the paper explicitly include one of the terms "spillover/complement*/substit*" to identify papers where information could be gleaned about this relationship even if it was not the primary purpose of the study. The final search term algorithm included (marijuana/marihuana/cannab*/pot/weed/THC) and (medical/nonmedical/recreat*/"adult-use"/decrim*/policy/ policies/liberal*/law/legal*) and (alcohol/drink*/beer/ethanol/etoh/wine/spirit*/liquor).

Inclusion Criteria

Two senior researchers independently screened all titles and abstracts to identify articles for exclusion because they were reviews, commentaries, or descriptive in nature, or because they did not include an outcome clearly identified as related to alcohol. Studies deemed eligible by at least one reviewer were included for full-text review and assigned to one of the authors of this report to read, assess for methodological strengths of

the study, and extract data for coding of studies. At this stage, additional articles were excluded if (1) the study did not examine the effect of a change in cannabis policy on an alcohol-specific outcome, which is the same criteria used by Guttmannova et al.;[31] or (2) the study did not use a methodologically appropriate design for the identification of plausibly causal policy impacts on the alcohol-related outcome. Methodological designs deemed inappropriate for identification of policy effects were those that either had no within-state or out-of-state control group or did not use a pre-post analytic design.

Data Extraction

A standardized Excel form was used to extract information by each reviewer on the details of the reviewed papers, including the study's data source(s); years covered; policy measures and sources; population included; methods used; specific alcohol-related, cannabis-related, and other outcome measures examined; statistical significance and magnitude of findings; and study limitations.

Quality Assessment

Although inclusion criteria restricted the sample to studies that are methodologically strong in terms of use of a comparison or control group and use of pre- and post-policy evaluation design, additional aspects of these studies are important for considering the reliability of the findings. First, consideration of the data set used for identification of findings is important as studies with state-representative samples—such as the National Survey on Drug Use and Health,[39] the state-specific Youth Risk Behavior Surveillance System,[40] and several state-specific school surveys—produce more reliable findings than data sources that do not consist of state-representative samples for all states, such as the Monitoring the Future[41] survey.[42,43] Second, the time period in which policies are evaluated is important as it can influence which states provide variation to identify policy effects, and states that adopted policies prior to 2010 were far more lenient in terms of market regulation than were states that have adopted policies since then.[44] Third, the specific cannabis policy being evaluated might matter, as well as the specific policy dimensions, as these policies may influence use through different mechanisms, including norms, availability/access, and/or price.[25] Fourth, as mentioned already, many studies fail to include a measure for alcohol policy over the same time period or may include what the literature has deemed a relatively weak measure of the overall alcohol policy environment, which might generate omitted variable bias in the analysis.[36] Finally, the authors considered the reliability of the finding not just in terms of the significance and directionality of findings for alcohol but in terms of cannabis as well. For example, a policy that was associated with a significant decrease in alcohol use without a corresponding significant increase in cannabis use seems unlikely to truly reflect substitution between the two substances. All of these aspects were considered when assessing the actual findings from each study.

Search Results

The search identified 3,446 unique monographs (Figure 2). Title and abstract screening led to the exclusion of the majority of the identified articles (*N* = 3,288). The remaining 158 articles underwent full-text assessment, from which 23 were included in this review.

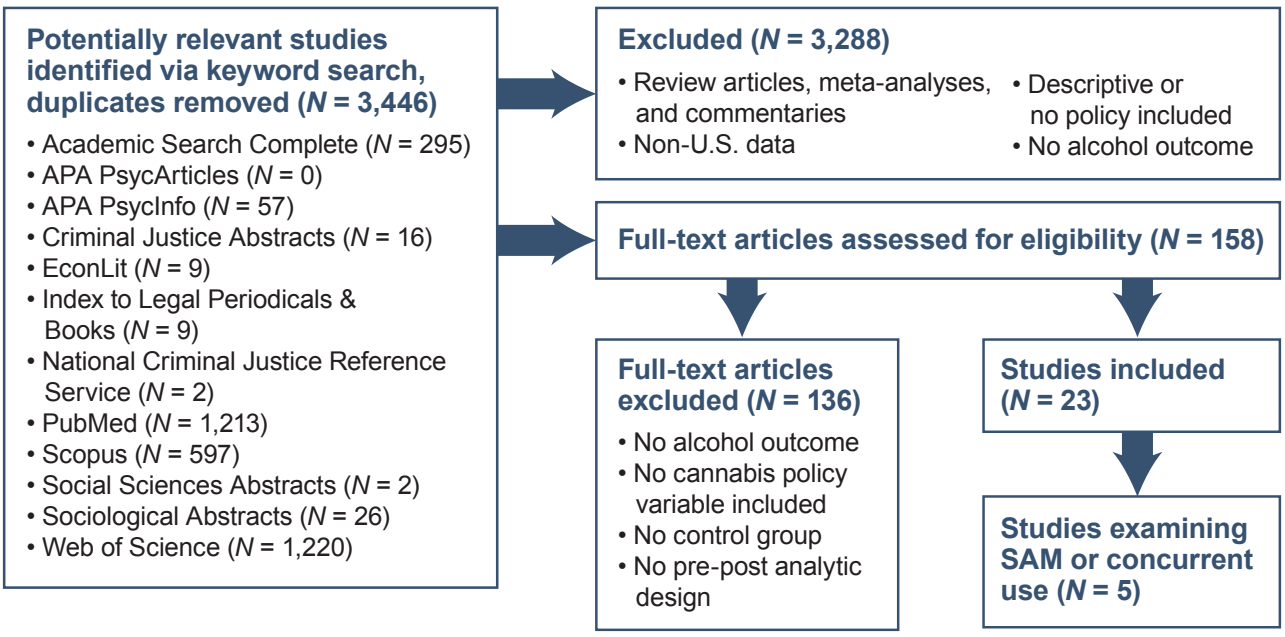

Figure 2. Flow diagram showing search algorithm results and inclusion/exclusion process to generate final studies included in this review. *Note:* APA, American Psychological Association; SAM, simultaneous use of alcohol and marijuana.

Results

Table 1 summarizes key characteristics of the 23 included studies. Study time frames span from 1977 to 2018, with study periods ranging from 4 to 39 years, and most studies (*n* = 15) included data from all or most U.S. states. Medical marijuana laws (MMLs) were the most common policy of interest (16 studies), followed by recreational marijuana laws (RMLs) (11 studies) and decriminalization (four studies). Seven of the 16 MML studies evaluated specific legal provisions (e.g., allowance for home cultivation) or implementation features (e.g., dispensaries operational); specific provisions of decriminalization and RMLs were not assessed, with the exception of the study by Hansen et al.,[28] who defined RML policy timing based on retail store availability. Regarding alcohol-related outcomes, most studies evaluated cannabis policy effects on self-reported prevalence or frequency of use (*n* = 10) or heavy or binge drinking behavior (variously defined across studies; *n* = 8), with relatively fewer studies evaluating alcohol-related driving or traffic fatality outcomes (*n* = 5), health care service utilization (*n* = 3), or sales data (*n* = 2).

Seven studies provided estimates specific to youth populations (generally adolescents no older than high school seniors), and six provided estimates for young adults (generally those who have entered college or are of college age, between the ages of 18 and 29). Many studies focusing on the impact of cannabis policies on cannabis use have found differing effects of these policies by age group; youth prevalence rates have generally been found to be insensitive to cannabis policies,[44,45] whereas prevalence rates in young adult and adult populations have generally been found to be positively associated with these cannabis liberalization laws.[18,44] This review's findings on the impact of cannabis policy on alcohol use across studies are reported by age group.

Cannabis Policies and Alcohol Use by Youth and Young Adults

Table 2 summarizes findings on the impact of medical and recreational cannabis policies on alcohol use as well as the key characteristics of the 13 included studies that assessed youth and/or young adult populations. Outcomes for measures of alcohol and cannabis use are reported in terms of direction and statistical significance in the final two columns. Those results that meet the standard threshold of statistical significance (alpha = 0.05 for a two-tailed test) are shown in bold.

Among the youth and young adult populations studied, findings regarding measures of use were inconsistent across data sets and studies, with some studies showing an increase in 30-day alcohol use with medical cannabis laws[46,47] and others showing a decrease.[48,49] Similarly, some studies noted an increase in binge drinking[48] whereas others detected a decrease in binge drinking.[49,50]

Findings for youth

Before interpreting these mixed results for youth by considering several factors (e.g., the measure of cannabis policy, years being evaluated, data sets used, inclusion of other alcohol policy measures), it is important to first note that the findings of the impact of these same policies on cannabis use from the same study (e.g., same population, same measure, same time period) were similarly inconclusive. Most of the studies showed that the impact of cannabis policies on cannabis use was statistically insignificant for youth, with few exceptions: Wen et al. suggested that cannabis liberalization was negatively associated with age at first use (i.e., more liberal policies were associated with earlier initiation), and that retail dispensaries specifically increased past-month use.[48] Cerdá et al. determined that MMLs were negatively associated with marijuana use among 8th graders only,[50] and Bailey et al. suggested that RMLs were positively associated with cannabis use in the past year.[51] These exceptions do not tell a consistent story of the impact of cannabis policy on cannabis use among youth and reinforce conclusions from earlier literature reviews.[25]

Given the inconsistency in cannabis policy effects on measures of cannabis use among youth, and that most of these studies detected no significant impact of the policy on cannabis use at all, it makes sense to focus on studies determining that a given measure influences cannabis use before trying to infer the measure's impact on alcohol use. A couple of studies showed significant impacts of cannabis policy on alcohol use—consistently suggesting that liberalization of these policies reduced alcohol use by youth.[49,52] In these studies, however, the policy was not significantly associated with cannabis use, with the exception of the study by Johnson et al., where the association was negative.[49] Only Bailey et al. found evidence of statistically significant increases in both past-year cannabis use and alcohol use,[51] with these results suggesting that cannabis and alcohol are economic complements. However, this study did not control for alcohol policy, raising concerns that this finding may be a function of an omitted variable bias.

Findings specific to RML, which is enacted only in states that have already passed MML, were also inconsistent. Coley et al. found a decrease in past-month cannabis use and level of alcohol use,[46] whereas Mason et al. detected an increase in cannabis use and a decrease in alcohol use,[52] and Bailey et al. showed increases in both cannabis and alcohol use.[51] The differences in findings are likely a function of the time periods examined, controls being included, and states sampled. Mason et al.[52] and Bailey et al.[51] similarly focused only on RML policies in just a few states but evaluated very different pre-period trends, while Coley et al.[46] covered a long time period similar to that of Bailey et al.[51] but also considered the influence of adopting MML and decriminalization statutes as well as included data from all 50 states.

Table 1. Summary of Characteristics of 23 Included Studies

Article	Study Period	Analytic Unit	Population and Age Group(s)	MJ Policy Measure(s)	Setting	Alcohol Outcomes
Wen et al. (2015)[48]	2004–2012	Individual	Youth (ages 12–20) Adults (age 21+)	• MML*	United States (all states)	• 30 d alcohol use • Heavy or binge use • AUD • CAM use • SAM use
Mason et al. (2016)[52]	2010–2013	Individual	Youth (grades 8–9)	• RML	Washington State	• Initiation • 30 d alcohol use
Dills et al. (2017)[47]	1977–2015	Individual	Youth (grade 12)	• Decrim • MML/RML	United States (48 states)	• 30 d alcohol use • Lifetime use • DUIA
Kerr et al. (2017)[55]	2012–2016	Individual	College students (ages 18–26)	• RML	Oregon, compared to unspecified number of states	• Heavy or binge use • CAM use
Sabia et al. (2017)[26]	1990–2012	Individual	All (age 18+) and some age-specific brackets	• Decrim • MML	United States (all states)	• 30 d alcohol use • Heavy or binge use
Cerdá et al. (2018)[50]	1991–2015	Individual	Youth (grades 8, 10, and 12)	• MML	United States (48 states)	• Heavy or binge use
Johnson et al. (2018)[49]	1991–2011	Individual	Youth (grades 9–12)	• MML*	United States (45 states)	• 30 d alcohol use • Heavy or binge use • CAM use
Kerr et al. (2018)[54]	2008–2016	Individual	College students (ages 18–26)	• RML	Oregon, compared to non-RML states	• 30 d alcohol use
Sabia & Nguyen (2018)[57]	1990–2014	Individual	Adults (ages 18–64) and some age-specific brackets	• Decrim • MML*	United States (all states)	• 30 d alcohol use
Steinemann et al. (2018)[69]	1993–2015	State	All	• MML	Hawaii	• Crash fatalities with alcohol
Andreyeva & Ukert (2019)[60]	1993–2013	Individual	Adults (ages 18+)	• MML*	United States (all states)	• Heavy or binge use
Delling et al. (2019)[63]	2010–2014	State	All	• RML	Colorado vs. New York & Oklahoma	• Hospitalizations
Dragone et al. (2019)[58]	2010–2014	County/MSA; 3-year averages	All (age 12+)	• MML • RML	Washington vs. Oregon	• Any use • Heavy or binge use

Table 1. Summary of Characteristics of 23 Included Studies (Continued)

Article	Study Period	Analytic Unit	Population and Age Group(s)	MJ Policy Measure(s)	Setting	Alcohol Outcomes
Meinhofer et al. (2019)[62]	2002–2014	State	Pregnant women (ages 12–49)	• MML	United States (48 states)	• Treatment admissions
Alley et al. (2020)[56]	2008–2018	Individual	College students (ages 18–26)	• RML	United States (48 states)	• Heavy or binge use
Baggio et al. (2020)[27]	2006–2015	County	All	• Decrim • MML* • RML	United States (48 states)	• Sales
Bailey et al. (2020)[51]	2002–2011, 2015–2018	Individual	Youth (under age 21)	• RML	Washington & Oregon	• Any use
Coley et al. (2020)[46]	1999–2017	Individual	Youth (mostly ages 14–18)	• Decrim • MML • RML	United States (47 states)	• 30 d alcohol use
Conyers & Ayres (2020)[64]	2010–2016	5-digit ZIP code	All	• MML*	Arizona	• ED visits
Cook et al. (2020)[53]	2010–2017	City	All (age 15+); ages 15–24; ages 25–44	• Decrim • MML	United States (large cities without MML or decrim by 2010)	• Alcohol-involved crash fatalities
Fink et al. (2020)[61]	1991–1992, 2001–2002, 2012–2013	Individual	Adults (age 18+)	• MML	United States (39 states)	• DUIA • DUIAM
Hansen et al. (2020)[28]	2000–2016	State	All (age 16+)	• RML*	Washington and Colorado, compared to non-RML states	• Crash fatalities
Veligati et al. (2020)[68]	1990–2016	State	All (age 14+)	• MML* • RML	United States (50 states)	• Sales

*Indicates if estimates were obtained for specific provisions of the laws (e.g., allowance for home cultivation) or implementation aspects (e.g., retail stores open).

Note: AUD, alcohol use disorder; CAM, concurrent alcohol and marijuana; Decrim, decriminalization; DUIA, driving under the influence of alcohol; DUIAM, driving under the influence of alcohol and marijuana; ED, emergency department; MJ, marijuana; MML, medical marijuana law; MSA, metropolitan statistical area; RML, recreational marijuana law; SAM, simultaneous alcohol and marijuana.

Table 2. Summary of Findings on Impact of Cannabis Policy on Alcohol Use Measures Among Youth and Young Adults

Article	Data (Study Period)	Age Group	MJ Policy Measure	Alcohol Policy or Price Measure	Impact of MJ Policy on Alcohol Use Measure	Impact of MJ Policy on MJ Use Measure
Youth Population (Age 19 and Younger)						
Wen et al. (2015)[48]	NSDUH (2004–2012)	Ages 12–20	MML	Beer tax	↓ 30 d total alcohol drinks ↑ 30 d binge drinking days ↓ AUD past year	↓ 30 d MJ use ↓ 30 d daily/near daily MJ use ↓ Days of MJ use ↑ **First MJ use last year** ↓ *DSM-IV* MJ use/dependence
			MML provisions	Beer tax	NS	Retail dispensary: ↑ **30 d MJ use**
Cerdá et al. (2018)[50]	MTF (1991–2015)	Grades 8, 10, and 12	MML	Beer tax	↓ **Binge drinking, 8th graders** ↑ Binge drinking, 10th graders ↑ Binge drinking, 12th graders	↓ **30 d MJ use for 8th graders** ↑ 30 d MJ use for 10th graders ↓ 30 d MJ use for 12th graders
Dills et al. (2017)[47]	MTF (1977–2015)	Grade 12	Either MML or RML	Beer tax, MLDA, Zero tolerance law, 0.08 BAC limit	↓ Lifetime alcohol use ↓ 30 d alcohol use ↓ 30 d number of times used	↓ Lifetime MJ use ↓ 30 d MJ use ↓ 30 d number of times used
			Either MML, RML, or Decrim	Beer tax, MLDA, Zero tolerance law, 0.08 BAC limit	↑ Lifetime alcohol use ↑ 30 d alcohol use ↓ 30 d number of times used	↑ Lifetime MJ use ↑ 30 d MJ use ↑ 30 d number of times used
Johnson et al. (2018)[49]	YRBSS (1991–2011)	Grades 9–12	MML	None	↓ **30 d alcohol use** ↓ 30 d alcohol use without MJ ↓ 30 d binge drinking	↓ 30 d MJ use without alcohol
			MML Restrictiveness (Scale: Less restrictive law means higher value)	None	↓ **30 d alcohol use** ↓ **30 d alcohol use without MJ** ↓ **30 d binge drinking**	↓ 30 d MJ use without alcohol
Coley et al. (2020)[46]	YRBSS (1999–2017)	Ages 14–18+	RML	Beer tax	↓ Any 30 d alcohol use ↓ Level of 30 d alcohol use	↑ Any 30 d MJ use ↓ **Level of 30 d MJ use**
			MML	Beer tax	↑ Any 30 d alcohol use ↓ Level of 30 d alcohol use	↑ Any 30 d MJ use ↓ Level of 30 d MJ use
			Decrim	Beer tax	↑ Any 30 d alcohol use ↓ Level of 30 d alcohol use	↑ Any 30 d MJ use ↓ Level of 30 d MJ use
Mason et al. (2016)[52]	Common Sense Parenting Intervention (2010–2013)	Grades 8–9	RML	None	↓ **30 d alcohol use**	↑ 30 d MJ use

Table 2. Summary of Findings on Impact of Cannabis Policy on Alcohol Use Measures Among Youth and Young Adults (Continued)

Article	Data (Study Period)	Age Group	MJ Policy Measure	Alcohol Policy or Price Measure	Impact of MJ Policy on Alcohol Use Measure	Impact of MJ Policy on MJ Use Measure
Bailey et al. (2020)[51]	Seattle Social Development Project (2002–2011, 2015–2018)	≤ age 20	RML	None	↑ **Past-year alcohol use**	↑ **Past-year MJ use**
Young Adult Population (Ages 18-25)						
Kerr et al. (2017)[55]	Healthy Minds Study (2012–2016)	Ages 18–26	RML	No	↓ Past 2-week heavy alcohol use	↑ 30 d MJ use
Cook et al. (2020)[53]	FARS (2010–2017)	Ages 15–24	DML	Beer tax ALR laws	NS: MVCs with BAC ≥ 0.08%	None
			MML	Beer tax ALR laws	↓ MVCs with BAC ≥ 0.08%	None
Alley et al. (2020)[56]	National College Health Assessment (2008–2018)	Ages 18–26	RML	No	↓ Binge drinking ≤ 20 years ↓ **Binge drinking ≥ 21 years**	None
Sabia et al. (2017)[26]	BRFSS (1993–2012)	Ages 18–24	MML	Alcohol tax Zero tolerance laws	↓ **30 d any alcohol use** ↓ **30 d binge drinking**	None
Sabia & Nguyen (2018)[57]	BRFSS (1990–2016)	Ages 18–19 Ages 20–29	MML	Beer tax	↓ 30 d number of drinks, ages 18–19 ↓ **30 d number of drinks, ages 20–29**	None
Kerr et al. (2018)[54]	National College Health Assessment II (2008–2016)	Ages 18–26	RML	No	↓ 30 d alcohol use	↑ **30 d MJ use** ↑ **Level of 30 d MJ use**

Bold text indicates finding statistically significant at alpha = 0.05 for a two-tailed test.

Note: ALR, administrative license revocation; AUD, alcohol use disorder; BAC, blood alcohol concentration; BRFSS, Behavioral Risk Factor Surveillance System; Decrim, decriminalization; DML, decriminalization marijuana law; *DSM-IV*, *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition*; FARS, Fatality Analysis Reporting System; MJ, marijuana; MLDA, minimum legal drinking age; MML, medical marijuana law; MTF, Monitoring the Future; MVCs, motor vehicle crashes; NS, nonsignificant; NSDUH, National Survey on Drug Use and Health; RML, recreational marijuana law; YRBSS, Youth Risk Behavior Surveillance System.

Alcohol policies, with the exception of beer taxes, generally were ignored in the youth-focused studies in Table 2. In the one study that included a broader set of alcohol policies in the mix, the findings regarding alcohol and cannabis use were statistically insignificant.[47]

The fact that these seven well-designed studies generated inconsistent and generally insignificant results regarding the impact of cannabis policy on alcohol (as well as cannabis) use, leads the authors to conclude that the current state of the science regarding the impact of cannabis policy on youth cannabis and alcohol use is inconclusive. This is not to say that there is no relationship between cannabis policy and alcohol use, however, as the authors do not believe there are enough scientifically robust studies to draw such a conclusion using state-representative samples (i.e., National Survey on Drug Use and Health[39] and Youth Risk Behavior Surveillance System[40]) with strong alcohol policy controls included.

Findings for young adults

Table 2 also includes results from six studies that specifically assessed young adult populations. Across these studies a bit more consistency exists in that all studies in this group showed a negative association between RML/MML and past-month drinking and/or binge drinking. At least one study also showed a negative association between MML and alcohol-involved motor vehicle crashes (for accidents involving at least one individual with a blood alcohol concentration greater than 0.08%).[53] However, only two of the studies looked at the effects of cannabis policies on cannabis use within the population directly. One of the studies yielded a positive association between RML and cannabis use,[54] and the other study yielded a statistically insignificant result.[55] If cannabis liberalization policies do not directly influence cannabis use measures among the young adult population, it calls into question any causal association between liberalized cannabis policies and reduced alcohol use measures, at least with respect to a substitution hypothesis.

Two studies examining RML specifically showed a negative association between cannabis liberalization policy and heavy or binge drinking.[55,56] Only the study with an insignificant association provided evidence supporting a potentially causal relationship due to direct effects on cannabis, but it examined regular alcohol use and did not include any controls for alcohol policies.[54] All three studies that examined the effect of MML on young adult alcohol use included some measure of alcohol policy,[26,53,57] and two of these studies[26,57] showed statistically significant impacts on drinking. However, as noted already, none of the studies showed a positive association between MML policy and cannabis use.

Thus, while the findings across studies for young adults indicate a more consistent association between cannabis liberalization policies and alcohol use (one supporting possible substitution), the authors do not believe the evidence in total supports the conclusion that alcohol and cannabis are substitutes for this age group. There remain too many limitations of the existing literature to support such a robust conclusion, particularly in light of evidence showing higher prevalence of simultaneous use.[19]

Cannabis Liberalization Policy and Adult Alcohol Use

Table 3 reports the same information as Table 2, but focuses on the 14 included studies that reported results for the entire adult population. Given differences in the types of data assessed across these 14 studies, this paper considers the results by source of data. In other words, this review looks first at findings from studies using data from self-reported use, next examines findings from studies focusing on populations seeking care from the health system, then considers findings from studies using alcohol sales data, and finally considers results obtained from crash data.

Findings from self-reported use in population surveys

The authors identified two studies in this group that presented findings of the impact of cannabis policy on cannabis use as well as alcohol use, and both studies reported that more liberal cannabis policies were associated with increased past-month cannabis use and near-daily use.[48,58] However, these same two studies showed completely different impacts of their cannabis policy variable on past-month binge drinking, with Wen et al. noting an increase in past-month binge drinking days,[48] and Dragone et al. reporting a decrease in past-month binge drinking.[58] The difference across the two studies for alcohol use, despite similar findings for cannabis use, is likely driven by a few factors, including different cannabis policies being considered (MML and RML), different time periods being examined (2004–2012 and 2010–2014), and differences in the inclusion of alcohol policy measures (beer tax and none).

Three studies used data from the Behavioral Risk Factor Surveillance System (BRFSS),[59] albeit examining slightly different years and adult age groups.[26,57,60] All three studies suggested that alcohol use decreased with more liberal MML laws, although none of these studies considered the direct impact of the cannabis laws on adult cannabis use. Moreover, the two BRFSS studies that included alcohol policy measures in addition to MMLs generally showed statistically insignificant results except for binge drinking among adults age 35 and older.[26,57]

The last study examining self-reported use measures in survey data provided no further clarity on the relationship, as none of the results were statistically significant, although the outcome measures used in this study were driving under the influence of alcohol or cannabis, not use in the past month or year.[61]

Table 3. Summary of Findings on Impact of Cannabis Policy on Alcohol Use Measures Among Studies Examining Adult Populations or All Ages

Article	Data (Study Period)	Age Group	MJ Policy Measure	Alcohol Policy or Price Measure	Impact of MJ Policy on Alcohol Use Measure	Impact of MJ Policy on MJ Use Measure
Wen et al. (2015)[48]	NSDUH (2004–2012) Individual	Age 21+	MML	Beer tax	↑ 30 d total alcohol drinks ↑ **30 d binge drinking days** ↑ AUD in past year	↑ **30 d MJ use** ↑ **30 d daily/near-daily MJ use** ↑ Days of MJ use ↑ First MJ use last year ↑ *DSM-IV* MJ use/dependence
			MML provisions	Beer tax	Nonspecific pain provision: ↑ **30 d binge drinking days**	Nonspecific pain provision: ↑ **30 d MJ use** ↑ **Daily/near-daily MJ use**
Dragone et al. (2019)[58]	NSDUH (2010–2014) Aggregated	Adult	RML	No	↓ Alcohol use ↓ **30 d binge drinking**	↑ **MJ use**
Sabia et al. (2017)[26]	BRFSS (1990–2012)	Age 25+	MML	Alcohol tax Zero tolerance laws	↓ **30 d any alcohol use, ages 25–34** ↓ 30 d any alcohol use, age 35+ ↓ Binge drinking, ages 25–34 ↓ **Binge drinking, age 35+**	None
Sabia & Nguyen (2018)[57]	BRFSS (1990–2016)	Ages 30–39 Ages 40–49 Ages 50–64	MML	Beer tax	↓ 30 d number of drinks, ages 30–39 ↓ 30 d number of drinks, ages 40–49 ↓ 30 d number of drinks, ages 50–64	None
Andreyeva & Ukert (2019)[60]	BRFSS (1993–2013)	Age 18+	MML	No	↓ **30 d heavy drinking** ↓ **30 d risky drinking** ↓ 30 d binge drinking	None
			MML with active dispensaries	No	↑ 30 d heavy drinking ↓ 30 d risky drinking ↓ 30 d binge drinking	None
Fink et al. (2020)[61]	NLAES (1991–1992), NESARC (2001–2002), NESARC-III (2012–2013)	Age 18+	MML	No	NS: Driving under influence of alcohol	↑ Driving under influence of MJ
Meinhofer et al. (2019)[62]	TEDS (2002–2014)	Ages 12–49	MML	Beer tax	↑ **Tx admissions of pregnant women involving alcohol** ↑ Tx admissions of nonpregnant women involving alcohol	↑ **Tx admissions of pregnant women involving MJ** ↑ Tx admissions of nonpregnant women involving MJ
Conyers & Ayres (2020)[64]	Hospital discharge data (2010–2016) Arizona only	All ages	MML	No	↑ ED visits for *ICD-9* alcohol abuse and poisoning	↑ **ED visits for *ICD-9* MJ abuse and poisoning**
Delling et al. (2019)[63]	Healthcare Cost and Utilization Project (2010–2014)	All ages	RML	No	↑ **Admissions for *ICD-9* alcohol abuse**	↑ **Admissions for *ICD-9* MJ abuse**

Table 3. Summary of Findings on Impact of Cannabis Policy on Alcohol Use Measures Among Studies Examining Adult Populations or All Ages (Continued)

Article	Data (Study Period)	Age Group	MJ Policy Measure	Alcohol Policy or Price Measure	Impact of MJ Policy on Alcohol Use Measure	Impact of MJ Policy on MJ Use Measure
Baggio et al. (2020)[27]	Nielsen retail scanner data (2006–2015)	Age 21+	MML	Beer tax	**↓ Alcohol sales**	None
Veligati et al. (2020)[68]	Per capita sales (1990–2016)	All ages	MML	Beer tax Legal BAC limit of 0.08	↓ Per capita alcohol sales	None
			RML	Beer tax Legal BAC limit of 0.08	↑ Per capita alcohol sales	None
Cook et al. (2020)[53]	FARS (2010–2017)	Ages 25–44	Decrim	Beer tax ALR	↓ MVCs with BAC ≥ 0.08%	None
			MML	Beer tax ALR	**↓ MVCs with BAC ≥ 0.08%**	None
Steinemann et al. (2018)[69]	FARS (1993–2015)	All ages	MML	No	NS: Positive for alcohol among fatally injured drivers tested NS: Positive for alcohol among all fatally injured drivers	**↑ Positive for MJ among fatally injured drivers tested** **↑ Positive for MJ among all fatally injured drivers**
Hansen et al. (2020)[28]	FARS (2000–2016)	Age 16+	RML	No	NS: Fraction of fatal accidents with 1+ alcohol-positive driver NS: Total alcohol-related fatalities per 1 billion VMT	NS: Fraction of fatal accidents with 1+ MJ-positive driver NS: Total MJ-related fatalities per 1 billion VMT

Bold text indicates finding statistically significant at alpha = 0.05 for a two-tailed test.

Note: ALR, administrative license revocation; AUD, alcohol use disorder; BAC, blood alcohol concentration; BRFSS, Behavioral Risk Factor Surveillance System; Decrim, decriminalization; *DSM-IV*, *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition*; ED, emergency department; FARS, Fatality Analysis Reporting System; *ICD-9*, *International Classification of Diseases: Ninth Revision*; MJ, marijuana; MML, medical marijuana law; MVCs, motor vehicle crashes; NESARC, National Epidemiologic Survey on Alcohol and Related Conditions; NLAES, National Longitudinal Alcohol Epidemiologic Survey; NS, nonsignificant; NSDUH, National Survey on Drug Use and Health; RML, recreational marijuana law; TEDS, Treatment Episode Data Set; Tx, treatment; VMT, vehicle miles traveled; YRBSS, Youth Risk Behavior Surveillance System.

Findings from populations seeking health care services

Three studies included in this review focusing on the general population drew on data from different sectors of the health care system, and yet all three studies suggested that changes in cannabis policies were associated with an increase in both cannabis-involved and alcohol-related health care utilization.[62-64] The time periods examined differed quite a bit across the studies. In addition, studies examined different types of health care utilization, such as emergency department visits from a single state; hospital admission data for individuals diagnosed with marijuana abuse criteria using codes from the *International Classification of Diseases 9th Revision* (*ICD-9*);[65] and treatment admissions from the Treatment Episode Data Set[66] that includes people meeting cannabis abuse criteria according to the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*),[67] which distinguishes between substance abuse and substance dependence. The finding of statistical significance for both cannabis- and alcohol-related outcomes within the same data set in two of the three studies is reassuring for interpreting the results for the alcohol-involved outcome, although only one study included a measure of alcohol policy in their model,[62] raising concerns again of omitted variable bias. These limitations aside, it is striking how different the suggested relationship between alcohol and cannabis (evidence of complementarity) is from these health care system data compared with the self-reported survey data (evidence suggesting substitution). The difference may be a function of the fact that those seeking health care services may represent a different, perhaps more at-risk, population than those reporting in household surveys (i.e., women who are pregnant, people at risk of an overdose, and/or those meeting *DSM-IV* criteria for alcohol or cannabis abuse).

Findings from sales data

Two studies included in this review focused on population aggregated sales data, either in terms of total aggregated sales of alcoholic beverages per capita[68] or in terms of Nielsen scanner data sales.[27] The findings from these two studies suggested that alcohol sales were lower in states that adopted MMLs. However, the findings in the study by Veligati et al., which also included additional alcohol policy measures that better captured the overall alcohol environment and covered a much longer time period, suggested that this association was not statistically significant.[68] Moreover, Veligati et al. suggested that states that further adopted adult-use cannabis policies subsequently had an increase in per capita alcohol sales;[68] Baggio et al. did not consider these subsequent changes.[27]

Findings from fatal crash statistics

Despite numerous examinations of the impact of cannabis liberalization policies on fatal alcohol-involved crashes, only three studies made it through this review screen.[28,53,69] The three studies focused on very different age groups, cannabis policies, and time periods, so it is perhaps not surprising that here, too, no clear conclusions can be drawn. Although Cook et al. included some measures for alcohol policy and separately evaluated the impact of decriminalization and medical cannabis policies, the study did not include evidence of the direct effect of these policies on cannabis-related driving fatalities.[53] Thus, it is unclear if the decline in motor vehicle crashes associated with more significant drinking (blood alcohol concentration ≥ 0.08%) represented a true substitution effect. Although both Steinemann et al.[69] and Hansen et al.[28] also considered cannabis-involved crashes, neither found a significant impact on alcohol-involved crashes. The lack of an association, however, may reflect an omitted variable bias caused by the lack of controls for alcohol policies. Only Steinemann et al. included years prior to 2000, thereby capturing impacts of the early adopting medical cannabis states (California, Oregon, and Washington).[69] The heterogeneity in study designs makes it unwise to conclude that the inconsistent findings are evidence of no impact of these policies, but the findings also demonstrate the need for more consistent approaches across studies.

Cannabis Policies and Simultaneous/Concurrent Use Outcomes

This paper identified only five studies, summarized in Table 4, that met the inclusion criteria and considered the impact of cannabis policy on concurrent or simultaneous use of alcohol and cannabis.[48,49,55,61,69] None of these studies fully accounted for alcohol policies, despite including explicit measures of alcohol use. The two studies examining concurrent use among youth populations showed that concurrent use of cannabis and alcohol use/binge drinking generally both declined with adoption of medical cannabis policies,[48,49] but the findings were only statistically significant in the Johnson et al. study,[49] which did not control for the alcohol policy environment. In the one study examining young adults, Kerr et al. found that concurrent use of cannabis and heavy alcohol use increased with adoption of recreational cannabis laws,[55] but again the study did not account for the alcohol policy environment. The remaining three studies that examined concurrent and simultaneous use for adult populations generally supported complementary findings (like those for the young adults);[48,61,69] however, there again were inadequate controls for alcohol policy, with only Wen et al. including a measure of the beer excise tax.[48] Given the limited number of studies and the clear methodological concern related to omitted variable bias, it would be unwise to draw a conclusion from these results.

Table 4. Summary of Findings on Impact of Cannabis Policy on Concurrent or Simultaneous Use of Alcohol and Cannabis

Article	Data (Years Analyzed)	Age Group	MJ Policy Measure	Alcohol Policy Measure	Impact on Measure of Concurrent (C) or Simultaneous (S) Drinking
Youth Population (Age 19 and Younger)					
Wen et al. (2015)[48]	NSDUH (2004–2012) Individual level data	Ages 12–20	MML MML provisions	Beer tax	↓ 30 d cannabis use and binge drinking (C) ↓ 30 d cannabis use while drinking (S)
Johnson et al. (2018)[49]	YRBSS (1991–2011)	Grades 9–12	MML	No	**↓ 30 d alcohol and cannabis use (C),** but no effect on 30 d cannabis use without alcohol or 30 d alcohol use without MJ
			MML restrictiveness (Scale: Less restrictive law means higher value)	No	**↓ 30 d alcohol and cannabis use (C),** but no effect on cannabis use without alcohol
Young Adult Population (Ages 18–26)					
Kerr et al. (2017)[55]	Healthy Minds Study/ College Students (2012–2016)	Ages 18–26	RML	No	**↑ 30 d MJ use among heavy alcohol users in past 2 weeks (C)**
Adult Population (All Ages ≥ 18)					
Fink et al. (2020)[61]	NLAES (1991–1992), NESARC (2001–2002), NESARC-III (2012–2013)	Ages 18+	MML	No	↑ Self-reported driving under the influence of alcohol and MJ (S)
Wen et al. (2015)[48]	NSDUH (2004–2012) Individual level data	Ages 21+	MML MML provisions	Beer tax	**↑ 30 d MJ use and binge drinking (C)** **↑ 30 d MJ use while drinking (S)** Nonspecific pain provision: **↑ 30 d MJ use and binge drinking (C)** **↑ 30 d MJ use while drinking (S)**
Steinemann et al. (2018)[69]	FARS (1993–2015)	All ages	MML	No	**↑ Alcohol impairment for THC-positive drivers vs. non-THC-positive drivers**

Bold text indicates finding statistically significant at alpha = 0.05 for a two-tailed test.

Note: (C), concurrent; FARS, Fatality Analysis Reporting System; MJ, marijuana; MML, medical marijuana law; NESARC, National Epidemiologic Survey on Alcohol and Related Conditions; NLAES, National Longitudinal Alcohol Epidemiologic Survey; NS, nonsignificant; NSDUH, National Survey on Drug Use and Health; RML, recreational marijuana law; (S), simultaneous; THC, delta-9-tetrahydrocannabinol; YRBSS, Youth Risk Behavior Surveillance System.

Discussion

Despite being more than 20 years into the U.S. states' experiment with medical cannabis and nearly a decade into the experiment with recreational cannabis, the scientific literature remains unclear as to the impact of these liberalization policies on alcohol use. Although the number of studies has grown substantially, even since the previous comprehensive 2016 review conducted by Guttmannova et al.,[31] there remains insufficient evidence—both in terms of quantity and quality—to conclude that cannabis policy liberalization in U.S. states is associated with either increases or decreases in alcohol use or alcohol-related outcomes. The lack of a clear or consistent association exists mainly for medical cannabis policies, whereas for recreational cannabis policies the principal issue is a relatively small number of studies meeting inclusion criteria. Regarding relationships between cannabis policies and the concurrent or simultaneous use of alcohol and cannabis, this review also found no clear indication of an association one way or another; primarily because only a small number of unique studies met the inclusion criteria. Overall, the findings in this review, although inclusive of more recent studies, are broadly consistent with earlier findings from Guttmannova et al.[31]

It is possible that the inconclusive findings are a reflection of the fact that there may not be a meaningful or detectable association between cannabis policy liberalization and alcohol use. However, as noted throughout this review, it is also possible that the inconclusive findings pertaining to MMLs may be partly related to inconsistencies in research methods. Even studies that would be considered methodologically strong by including a comparison group and pre-post policy design often excluded relevant indicators to fully capture changes in the alcohol policy environment as well as the cannabis policy environment. Studies trying to assess the impact of RMLs on alcohol use might not yet have had sufficient time to properly evaluate their effects, particularly given the lag in opening markets after laws have passed and for markets to mature. Furthermore, the complement versus substitute nature of the relationship between cannabis and alcohol might vary based on prevalence, intensity, and frequency of use, which at this point the scientific literature is too limited to assess for reasons already discussed.

Because associations were not conclusive, the authors of this review default to the null hypothesis that (at least currently) there is no meaningful relationship between cannabis liberalization policies and alcohol use outcomes. Of course, it may be that that this conclusion is due to this review's efforts to try to pool evidence from across very different user groups, outcomes, and policies. More systematic studies considering heterogeneous effects across these dimensions will need to be considered, such as recent work by Kim et al. in 2021.[70] As the literature expands, attention paid to consistency across these sorts of dimensions may generate different conclusions.

Limitations

This review has several limitations. First, it is possible that cannabis policy is related to alcohol use through other mechanisms not captured by this review. The authors have focused on studies that examined an association between cannabis policy and alcohol use through a specific mechanism in which cannabis use is a mediator. However, it is possible that changes in public perception, norms, and cannabis use have led to changes in cannabis policy. Studies that focus on this mechanism, and any others, would have been excluded from this review even if they also showed an impact on alcohol use. Another limitation is that this review's inclusion criteria required a given study to examine the link between cannabis policy and both cannabis and alcohol use. The authors recognize that researchers may present evidence of the impact of cannabis policy on cannabis use separately from the impact of cannabis policy on alcohol use. For example, although Alley et al. did not examine the impact of RML on cannabis use,[56] a companion paper by Bae and Kerr did find a positive association between RML and cannabis use using the same data set and time period.[71]

Recommendations for Future Research

Perhaps the greatest contribution of this work is its identification of several key limitations in the literature, which should be better addressed in future work. In particular, studies should test the presumed intermediary causal mechanism between cannabis policy change and alcohol use. Specifically, if the mechanism of a reduction in alcohol consumption due to liberalized cannabis policies is thought to be through a substitution of cannabis for alcohol use, then studies should examine changes in cannabis use as a possible mediator of relationships between cannabis policies and alcohol use outcomes. Failing that, studies should at least report the change in cannabis consumption among the study population.

This review found no studies that formally examined cannabis use as a mediator in the relationship between cannabis policies and alcohol use outcomes. Ideally, one would have large-scale individual-level longitudinal data that would allow for the estimation of such mediation requests with attention to the temporal/causal sequencing among use of the two substances. Although several individual-level longitudinal data sets measure substance use behaviors—including the Monitoring the Future survey,[41] the National Longitudinal Survey of Youth,[72] and most recently the Adolescent Brain Cognitive Development Study[73]—these data sets have the limitation of small sample sizes that do not support state-representative analyses, which can cause problems for evaluating state-level policies (see Dilley et al.[43] for a discussion of these issues and inconsistency in findings regarding policy effectiveness). Given that most studies rely on repeated cross-sectional surveys (e.g., National Survey on Drug Use and Health[39] and BRFSS[59]), future research may improve our understanding by testing whether those subgroups or

subpopulations that experience greater changes in alcohol use following cannabis liberalization also experience greater changes in cannabis use. In light of these issues with existing survey data, a richer understanding of the relationship between cannabis policy and alcohol use may be developed by synthesizing evidence from the types of causal inference studies reviewed here with evidence from high-quality epidemiological studies that may have smaller sample sizes but richer person-level data on changes in substance use patterns.

Another obvious limitation of the existing literature is that nearly half of the studies included in this review failed to assess the impact of the change in cannabis policy on cannabis consumption among the study population. Among those that did, many showed no impact of cannabis policy on cannabis consumption directly. Therefore, although alcohol use measures may have decreased around the time these cannabis policies were adopted, these data do not constitute clear evidence of substitution. Future research will need to consider nuanced measures of cannabis use, as frequency of use does not accurately reflect amount consumed overall or exposure to delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis. If liberalized cannabis policies impact the potency (i.e., THC concentration) or types of formulations or cannabinoids consumed,[74,75] then studies focused on frequency will miss important changes in use.

A third limitation is that most studies do not adequately account for alcohol policies, which are strongly related to alcohol consumption. Specifically, more than half of the studies did not account for any alcohol policies, and those that did typically accounted only for the specific excise tax for beer. Taxation is but one of many policies, and even in terms of tax policies, specific excise taxes (i.e., beer) represent only a small percentage of the total price of alcohol and are smaller in magnitude than other tax types that are applied to alcohol. Other policies affecting alcohol availability, such as outlet density or hours of sale, also may be important to include. Additionally, it may be important to consider social determinants of alcohol consumption, such as liberal state politics or religious affiliation that are statistically associated with political and/or social movements favoring the liberalization of cannabis policies that could result in associations between cannabis policies and alcohol consumption-related outcomes.

A fourth limitation is the treatment of RML and MML policies as monolithic across states, rarely examining state-level variations in policies, evaluating policy components or the timing of policies. It is not a trivial point to note that every state that adopted an RML policy prior to 2020 transitioned from a MML policy. It is possible that positive associations that were identified for RML policies in hospital admissions data[63] and/or aggregated sales data[68] may reflect the longer term impact of a mature market on the medical and recreational cannabis markets combined rather than an isolated impact of RML alone. Relatedly, they also might reflect a changing cannabis policy environment due to differential implementation caused by a specific federal response and/or changes in implementation that occur over time. Studies also may vary in their treatment of policy timing—whether the date for RML or MML corresponds to the date of passage, enactment, or implementation of the law (e.g., first day of retail sales), which may influence whether or not the policy is shown to have an impact on cannabis or alcohol use. Additionally, variation within states—for example, across municipalities that do or do not permit cannabis sales regardless of statewide policy—or differences in retail availability provide another opportunity for future research.

Future research will need to consider how the evolving cannabis state markets and federal position lead to changes in how a given law is interpreted by market participants, which will influence consumption of cannabis as well as any economic complement or substitute.

It is clear from the research evidence to date that the answer to the critical public health question regarding the impact of cannabis liberalization policies on alcohol use, particularly heavy drinking and drinking-related harm, remains unknown. Population evidence, such as showing that the prevalence of simultaneous use of alcohol and marijuana is increasing among those who consume high quantities of alcohol,[19] runs counter to conclusions often drawn from a few studies that alcohol and cannabis are economic substitutes. Like the previous comprehensive review published by Guttmannova et al.,[31] this review is unable to provide a singular interpretation of the scientific evidence to date, despite examining the more recent evidence, which has grown rapidly in the last 5 years. Significant methodological shortcomings need to be overcome before there is a clear answer to the nature of the relationship, and researchers will need to pay close attention as to whether the short-term response differs from the long-term relationship.

References

1. National Conference of State Legislatures. *State Medical Cannabis Laws*. 2021. https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx.
2. Caulkins JP, Kilmer B, Kleiman MAR. *Marijuana Legalization: What Everyone Needs to Know*. Oxford University Press; 2016.
3. Caulkins JP, Kilmer B, Kleiman MAR, et al. *Considering Marijuana Legalization: Insights for Vermont and Other Jurisdictions*. Santa Monica, CA: RAND Corporation; 2015.
4. Volkow ND, Baler RD, Compton WM, Weiss SR. Adverse health effects of marijuana use. *N Engl J Med*. 2014;370(23):2219-2227. https://doi.org/10.1056/nejmra1402309.
5. Campeny E, López-Pelayo H, Nutt D, et al. The blind men and the elephant: Systematic review of systematic reviews of cannabis use related health harms. *Eur Neuropsychopharmacol*. 2020;33:1-35. https://doi.org/10.1016/j.euroneuro.2020.02.003.
6. Griswold MG, Fullman N, Hawley C, et al., Global Burden of Diseases, Injuries, and Risk Factors 2016 Alcohol Collaborators. Alcohol use and burden for 195 countries and territories, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2018;392(10152):1015-1035. https://doi.org/10.1016/s0140-6736(18)31310-2.

7. Martinez P, Kerr WC, Subbaraman MS, Roberts SC. New estimates of the mean ethanol content of beer, wine, and spirits sold in the United States show a greater increase in per capita alcohol consumption than previous estimates. *Alcohol Clin Exp Res.* 2019;43(3):509-521. https://doi.org/10.1111/acer.13958.
8. Slater ME, Alpert HR, for National Institute on Alcohol Abuse and Alcoholism. *Apparent Per Capita Alcohol Consumption: National, State, and Regional Trends, 1977–2018*. Surveillance Report 115. 2020. https://pubs.niaaa.nih.gov/publications/surveillance115/CONS18.htm.
9. Grucza RA, Sher KJ, Kerr WC, et al. Trends in adult alcohol use and binge drinking in the early 21st-century United States: A meta-analysis of 6 National Survey Series. *Alcohol Clin Exp Res.* 2018;42(10):1939-1950. https://doi.org/10.1111/acer.13859.
10. National Academies of Sciences, Engineering, and Medicine. *The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research*. Washington, DC: The National Academies Press; 2017.
11. Pacula RL, Jacobson M, Maksabedian EJ. In the weeds: A baseline view of cannabis use among legalizing states and their neighbours. *Addiction.* 2016;111(6):973-980. https://doi.org/10.1111/add.13282.
12. Subbaraman MS, Kerr WC. Simultaneous versus concurrent use of alcohol and cannabis in the National Alcohol Survey. *Alcohol Clin Exp Res.* 2015;39(5):872-879. https://doi.org/10.1111/acer.12698.
13. Terry-McElrath YM, O'Malley PM, Johnston LD. Simultaneous alcohol and marijuana use among U.S. high school seniors from 1976 to 2011: Trends, reasons, and situations. *Drug Alcohol Depend.* 2013;133(1):71-79. https://doi.org/10.1016/j.drugalcdep.2013.05.031.
14. Agrawal A, Budney AJ, Lynskey MT. The co-occurring use and misuse of cannabis and tobacco: A review. *Addiction.* 2012;107(7):1221-1233. https://doi.org/10.1111/j.1360-0443.2012.03837.x.
15. Butterworth P, Slade T, Degenhardt L. Factors associated with the timing and onset of cannabis use and cannabis use disorder: Results from the 2007 Australian National Survey of Mental Health and Well-Being. *Drug Alcohol Rev.* 2014;33(5):555-564. https://doi.org/10.1111/dar.12183.
16. Haas AL, Wickham R, Macia K, Shields M, Macher R, Schulte T. Identifying classes of conjoint alcohol and marijuana use in entering freshmen. *Psychol Addict Behav.* 2015;29(3):620-626. https://doi.org/10.1037/adb0000089.
17. Midanik LT, Tam TW, Weisner C. Concurrent and simultaneous drug and alcohol use: Results of the 2000 National Alcohol Survey. *Drug Alcohol Depend.* 2007;90:72-80.
18. Hasin DS, Sarvet AL, Cerdá M, et al. US adult illicit cannabis use, cannabis use disorder, and medical marijuana laws: 1991-1992 to 2012-2013. *JAMA Psychiatry.* 2017;74(6):579-588. https://doi.org/10.1001/jamapsychiatry.2017.0724.
19. Terry-McElrath YM, Patrick ME. Simultaneous alcohol and marijuana use among young adult drinkers: Age-specific changes in prevalence from 1977 to 2016. *Alcohol Clin Exp Res.* 2018;42(11):2224-2233. https://doi.org/10.1111/acer.13879.
20. Terry-McElrath YM, O'Malley PM, Johnston LD. Alcohol and marijuana use patterns associated with unsafe driving among U.S. high school seniors: High use frequency, concurrent use, and simultaneous use. *J Stud Alcohol Drugs.* 2014;75(3):378-389. https://doi.org/10.15288/jsad.2014.75.378.
21. Yurasek AM, Aston ER, Metrik J. Co-use of alcohol and cannabis: A review. *Curr Addict Rep.* 2017;4:184-193. https://doi.org/10.1007/s40429-017-0149-8.
22. Hartman RL, Huestis MA. Cannabis effects on driving skills. *Clin Chem.* 2013;59(3):478-492. https://doi.org/10.1373/clinchem.2012.194381.
23. Ramaekers JG, Robbe HWJ, O'Hanlon JF. Marijuana, alcohol and actual driving performance. *Hum Psychopharmacol.* 2000;15(7):551-558. https://doi.org/10.1002/1099-1077(200010)15:7%3C551::aid-hup236%3E3.0.co;2-p.
24. Grossman M, Chaloupka FJ, Saffer H, Laixuthai A. Effects of alcohol price policy on youth: A summary of economic research. *J Res Adolesc.* 1994;4(2):347-364. https://doi.org/10.1146/annurev-clinpsy-032816-045128.
25. Pacula RL, Smart R. Medical marijuana and marijuana legalization. *Annu Rev Clin Psychol.* 2017;13:397-419. https://doi.org/10.1146/annurev-clinpsy-032816-045128.
26. Sabia JJ, Swigert J, Young T. The effect of medical marijuana laws on body weight. *Health Econ.* 2017;26(1):6-34. https://doi.org/10.1002/hec.3267.
27. Baggio M, Chong A, Kwon S. Marijuana and alcohol: Evidence using border analysis and retail sales data. *Can J Econ.* 2020;53(2):563-591. https://doi.org/10.1111/caje.12437.
28. Hansen B, Miller K, Weber C. Early evidence on recreational marijuana legalization and traffic fatalities. *Econ Inq.* 2020;58(2):547-568. https://doi.org/10.1111/ecin.12751.
29. Risso C, Boniface S, Subbaraman MS, Englund A. Does cannabis complement or substitute alcohol consumption? A systematic review of human and animal studies. *J Psychopharmacol.* 2020;34(9):938-954. https://doi.org/10.1177/0269881120919970.
30. Subbaraman MS. Substitution and complementarity of alcohol and cannabis: A review of the literature. *Subst Use Misuse.* 2016;51(11):1399-1414. https://doi.org/10.3109/10826084.2016.1170145.
31. Guttmannova K, Lee CM, Kilmer JR, et al. Impacts of changing marijuana policies on alcohol use in the United States. *Alcohol Clin Exp Res.* 2016;40(1):33-46. https://doi.org/10.1111/acer.12942.
32. Athey S, Imbens GW. The state of applied econometrics: Causality and policy evaluation. *J Econ Perspect.* 2017;31(2):3-32. https://doi.org/10.1257/jep.31.2.3.
33. Schlotter M, Schwerdt G, Woessmann L. Econometric methods for causal evaluation of education policies and practices: A non-technical guide. *Educ Econ.* 2011;19(2):109-137. https://doi.org/10.1080/09645292.2010.511821.
34. Park JY, Wu LT. Trends and correlates of driving under the influence of alcohol among different types of adult substance users in the United States: A national survey study. *BMC Public Health.* 2019;19(1):509. https://doi.org/10.1186/s12889-019-6889-8.
35. O'Hara RE, Armeli S, Tennen H. Alcohol and cannabis use among college students: Substitutes or complements? *Addict Behav.* 2016;58:1-6. https://doi.org/10.1016/j.addbeh.2016.02.004.
36. Naimi TS, Blanchette JG, Xuan Z, Chaloupka FJ. Erosion of state alcohol excise taxes in the United States. *J Stud Alcohol Drugs.* 2018;79(1):43-48. https://doi.org/10.15288/jsad.2018.79.43.
37. Xuan Z, Chaloupka FJ, Blanchette JG, et al. The relationship between alcohol taxes and binge drinking: Evaluating new tax measures incorporating multiple tax and beverage types. *Addiction.* 2015;110(3):441-450. https://doi.org/10.1111/add.12818.
38. Page MJ, McKenzie JE, Bossuyt PM, et al. Updating guidance for reporting systematic reviews: Development of the PRISMA 2020 statement. *J Clin Epidemiol.* 2021;134:103-112. https://doi.org/10.1016/j.jclinepi.2021.02.003.
39. Substance Abuse and Mental Health Services Administration (SAMHSA). National Survey on Drug Use and Health website. 2021. https://www.samhsa.gov/data/data-we-collect/nsduh-national-survey-drug-use-and-health.
40. Centers for Disease Control and Prevention (CDC). Youth Risk Behavior Surveillance System (YRBSS) website. No date. https://www.cdc.gov/healthyyouth/data/yrbs/index.htm.
41. National Institute on Drug Abuse (NIDA). Monitoring the Future Survey website. No date. https://www.drugabuse.gov/drug-topics/trends-statistics/monitoring-future.

42. Midgette G, Reuter P. Has cannabis use among youth increased after changes in its legal status? A commentary on use of Monitoring the Future for analyses of changes in state cannabis laws. *Prev Sci.* 2020;21(2):137-145. https://doi.org/10.1007/s11121-019-01068-4.
43. Dilley JA, Richardson SM, Kilmer B, Pacula RL, Segawa MB, Cerdá M. Prevalence of cannabis use in youths after legalization in Washington State. *JAMA Pediatr.* 2019;173(2):192-193. https://doi.org/10.1001/jamapediatrics.2018.4458.
44. Smart R, Pacula RL. Early evidence of the impact of cannabis legalization on cannabis use, cannabis use disorder, and the use of other substances: Findings from state policy evaluations. *Am J Drug Alcohol Abuse.* 2019;45(6):644-663. https://doi.org/10.1080/00952990.2019.1669626.
45. Sarvet AL, Wall MM, Fink DS, et al. Medical marijuana laws and adolescent marijuana use in the United States: A systematic review and meta-analysis. *Addiction.* 2018;113(6):1003-1016. https://doi.org/10.1111/add.14136.
46. Coley RL, Kruzik C, Ghiani M, Carey N, Hawkins SS, Baum CF. Recreational marijuana legalization and adolescent use of marijuana, tobacco, and alcohol. *J Adolesc Health.* 2021;69(1):41-49. https://doi.org/10.1016/j.jadohealth.2020.10.019.
47. Dills AK, Goffard S, Miron J. The effects of marijuana liberalizations: Evidence from Monitoring the Future. National Bureau of Economic Research working paper w23779. 2017. https://www.nber.org/papers/w23779.
48. Wen H, Hockenberry JM, Cummings JR. The effect of medical marijuana laws on adolescent and adult use of marijuana, alcohol, and other substances. *J Health Econ.* 2015;42:64-80. https://doi.org/10.1016/j.jhealeco.2015.03.007.
49. Johnson JK, Johnson RM, Hodgkin D, Jones AA, Matteucci AM, Harris SK. Heterogeneity of state medical marijuana laws and adolescent recent use of alcohol and marijuana: Analysis of 45 states, 1991-2011. *Subst Abus.* 2018;39(2):247-254. https://doi.org/10.1080/08897077.2017.1389801.
50. Cerdá M, Sarvet AL, Wall M, et al. Medical marijuana laws and adolescent use of marijuana and other substances: Alcohol, cigarettes, prescription drugs, and other illicit drugs. *Drug Alcohol Depend.* 2018;183:62-68. https://doi.org/10.1016/j.drugalcdep.2017.10.021.
51. Bailey JA, Epstein M, Roscoe JN, Oesterle S, Kosterman R, Hill KG. Marijuana legalization and youth marijuana, alcohol, and cigarette use and norms. *Am J Prev Med.* 2020;59(3):309-316. https://doi.org/10.1016/j.amepre.2020.04.008.
52. Mason WA, Fleming CB, Ringle JL, Hanson K, Gross TJ, Haggerty KP. Prevalence of marijuana and other substance use before and after Washington State's change from legal medical marijuana to legal medical and nonmedical marijuana: Cohort comparisons in a sample of adolescents. *Subst Abus.* 2016;37(2):330-335. https://doi.org/10.1080/08897077.2015.1071723.
53. Cook AC, Leung G, Smith RA. Marijuana decriminalization, medical marijuana laws, and fatal traffic crashes in US cities, 2010–2017. *Am J Public Health.* 2020;110(3):363-369. https://doi.org/10.2105/ajph.2019.305484.
54. Kerr DCR, Bae H, Koval AL. Oregon recreational marijuana legalization: Changes in undergraduates' marijuana use rates from 2008 to 2016. *Psychol Addict Behav.* 2018;32(6):670-678. https://doi.org/10.1037/adb0000385.
55. Kerr DCR, Bae H, Phibbs S, Kern AC. Changes in undergraduates' marijuana, heavy alcohol and cigarette use following legalization of recreational marijuana use in Oregon. *Addiction.* 2017;112(11):1992-2001. https://doi.org/10.1111/add.13906.
56. Alley ZM, Kerr DCR, Bae H. Trends in college students' alcohol, nicotine, prescription opioid and other drug use after recreational marijuana legalization: 2008-2018. *Addict Behav.* 2020;102:106212. https://doi.org/10.1016/j.addbeh.2019.106212.
57. Sabia JJ, Nguyen TT. The effect of medical marijuana laws on labor market outcomes. *J Law Econ.* 2018;61(3):361-396. https://doi.org/10.1086/701193.
58. Dragone D, Prarolo G, Vanin P, Zanella G. Crime and the legalization of recreational marijuana. *J Econ Behav Organ.* 2019;159:488-501. https://doi.org/10.1016/j.jebo.2018.02.005.
59. CDC. Behavioral Risk Factor Surveillance System website. No date. https://www.cdc.gov/brfss.
60. Andreyeva E, Ukert B. The impact of medical marijuana laws and dispensaries on self-reported health. *Forum Health Econ Policy.* 2019;22(2). https://doi.org/10.1515/fhep-2019-0002.
61. Fink DS, Stohl M, Sarvet AL, Cerda M, Keyes KM, Hasin DS. Medical marijuana laws and driving under the influence of marijuana and alcohol. *Addiction.* 2020;115(10):1944-1953. https://doi.org/10.1111/add.15031.
62. Meinhofer A, Witman A, Murphy SM, Bao YH. Medical marijuana laws are associated with increases in substance use treatment admissions by pregnant women. *Addiction.* 2019;114(9):1593-1601. https://doi.org/10.1111/add.14661.
63. Delling FN, Vittinghoff E, Dewland TA, et al. Does cannabis legalisation change healthcare utilisation? A population-based study using the healthcare cost and utilisation project in Colorado, USA. *BMJ Open.* 2019;9(5):e027432. https://doi.org/10.1136/bmjopen-2018-027432.
64. Conyers G, Ayres I. A lottery test of the effect of dispensaries on emergency room visits in Arizona. *Health Econ.* 2020;29(8):854-864. https://doi.org/10.1002/hec.4013.
65. World Health Organization (WHO). *International Classification of Diseases: Ninth Revision. Basic Tabulation List With Alphabetical Index.* Geneva, Switzerland: WHO; 1978. https://apps.who.int/iris/handle/10665/39473.
66. SAMHSA. Treatment Episode Data Set (TEDS) website. No date. https://www.samhsa.gov/data/data-we-collect/teds-treatment-episode-data-set#teds_d.
67. American Psychiatric Association (APA). *Diagnostic and Statistical Manual of Mental Disorders.* 4th ed. Arlington, VA: APA; 1994.
68. Veligati S, Howdeshell S, Beeler-Stinn S, et al. Changes in alcohol and cigarette consumption in response to medical and recreational cannabis legalization: Evidence from U.S. state tax receipt data. *Int J Drug Policy.* 2020;75:102585. https://doi.org/10.1016/j.drugpo.2019.10.011.
69. Steinemann S, Galanis D, Nguyen T, Biffl W. Motor vehicle crash fatalities and undercompensated care associated with legalization of marijuana. *J Trauma Acute Care Surg.* 2018;85(3):566-571. https://doi.org/10.1097/ta.0000000000001983.
70. Kim JH, Weinberger AH, Zhu J, Barrington-Trimis J, Wyka K, Goodwin RD. Impact of state-level cannabis legalization on poly use of alcohol and cannabis in the United States, 2004–2017. *Drug Alcohol Depend.* 2021;218:108364. https://doi.org/10.1016/j.drugalcdep.2020.108364.
71. Bae H, Kerr DCR. Marijuana use trends among college students in states with and without legalization of recreational use: Initial and longer-term changes from 2008 to 2018. *Addiction.* 2020;115(6):1115-1124. https://doi.org/10.1111/add.14939.
72. Bureau of Labor Statistics, U.S. Department of Labor. *National Longitudinal Survey of Youth 1979.* Columbus, OH: Center for Human Resource Research, The Ohio State University; 2019. https://www.nlsinfo.org/content/cohorts/nlsy79.
73. ABCD Research Consortium. Adolescent Brain Cognitive Development (ABCD) Study website. No date. https://abcdstudy.org.
74. Sevigny EL, Pacula RL, Heaton P. The effects of medical marijuana laws on potency. *Int J Drug Policy.* 2014;25(2):308-319.
75. Davenport SS, Caulkins JP. Evolution of the United States marijuana market in the decade of liberalization before full legalization. *J Drug Issues.* 2016;46(4):411-427. https://doi.org/10.1177%2F0022042616659759.

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2022;42(1):04 | https://doi.org/10.35946/arcr.v42.1.04 Published: 10 February 2022

Patterns of Cannabis and Alcohol Co-Use: Substitution Versus Complementary Effects

Rachel L. Gunn,[1] Elizabeth R. Aston,[1] and Jane Metrik[1,2]

[1]Center for Alcohol and Addiction Studies, Brown University School of Public Health, Providence, Rhode Island
[2]Providence VA Medical Center, Providence, Rhode Island

Correspondence
Correspondence regarding this article should be directed to Rachel Gunn, Ph.D., Center for Alcohol and Addiction Studies, Brown University, Box G-S121-5, Providence, RI 02912. Email: Rachel_Gunn@brown.edu

Acknowledgments
This work was supported by National Institute on Alcohol Abuse and Alcoholism grants R01AA024091 and K08AA027551; National Institute on Drug Abuse grant K01DA039311; and National Institute of General Medical Sciences COBRE grant P20GM130414.

Disclosures
The authors declare no competing financial or nonfinancial interests.

PURPOSE: The purpose of this review is to discuss the literature regarding the concurrent use (co-use) of alcohol and cannabis and competing hypotheses as to whether cannabis acts as a substitute for (i.e., replacing the effects of alcohol, resulting in decreased use) or a complement to (i.e., used to enhance the effects of alcohol, resulting in increased use) alcohol. The impact of cannabis use on alcohol-related outcomes has received increased attention in the wake of ongoing legalization of cannabis for both medical and recreational purposes. Evidence for both hypotheses exists in the literature across a broad range of data collection methods and samples and is carefully reviewed here. In addition, various mechanisms by which cannabis may act as an alcohol substitute or complement are explored in depth with the goal of better understanding equivocal findings.
SEARCH METHODS: This review includes articles that were identified from a search for studies on alcohol and cannabis co-use, with a specific focus on studies exploring complementary versus substitution aspects of co-use. Search terms were included in Google Scholar, PsycINFO, MEDLINE, and Web of Science. Eligible studies were those that measured alcohol and cannabis co-use in human samples in laboratory, survey, or ecological momentary assessment studies, or that directly referenced substitution or complementary patterns of use.
SEARCH RESULTS: Search results returned 650 articles, with 95 meeting inclusion criteria.
DISCUSSION AND CONCLUSIONS: Results of this review reveal compelling evidence for both substitution and complementary effects, suggesting nuanced yet significant distinctions across different populations examined in these studies. Several mechanisms for the impact of cannabis use on alcohol-related outcomes are identified, including patterns and context of co-use, timing and order of use, cannabinoid formulation, pharmacokinetic interactions, and user characteristics (including diagnostic status), all of which may influence substitution versus complementary effects. This review will inform future research studies examining this topic in both clinical and community samples and aid in the development of treatment and prevention efforts targeting those populations most vulnerable to negative consequences of co-use. Finally, this review highlights the need for additional research in more diverse samples and the use of mixed-methods designs to examine both pharmacological and contextual influences on co-use.

Keywords: alcohol; cannabis; marijuana; concurrent use; co-use

Use of alcohol and related problems cause significant global and individual health-related harms, and alcohol use is currently the third-leading cause of preventable death in the United States.[1,2] Alcohol and cannabis are among the most commonly used psychoactive substances in the United States.[3] Although concurrent use of alcohol and cannabis (i.e., co-use: defined as using both substances, but not necessarily so that their effects overlap) has been linked to increased alcohol consumption and alcohol-related consequences compared to single substance use,[4-6] findings as to whether cannabis use contributes to or reduces alcohol-related harms are mixed. In particular, reviews of this topic have identified competing theories, namely whether cannabis acts as a substitute (i.e., replacing the effects of alcohol, resulting in decreased use) or a complement (i.e., used to enhance the effects of alcohol, resulting in increased use).[7-9] Here, the current literature is reviewed, and potential mechanisms whereby cannabis use is associated with alcohol-related behaviors and their substitution versus complementary effects are discussed. There is also a relevant distinction between concurrent use (i.e., co-use) and simultaneous use (i.e., using both substances together so that the effects overlap), which is linked to a unique set of consequences in adult and adolescent samples.[6,10] The current review focuses on co-use generally and the impact of cannabis use specifically on alcohol use and related outcomes, whereas the topic of simultaneous use is reviewed elsewhere in this topic series.[11]

Search Methods

A literature search was conducted to identify articles for this review via Google Scholar, PsycINFO, MEDLINE, and Web of Science to identify studies that examined whether cannabis acts as a substitute for or complement to alcohol use and related outcomes. Search terms used were (1) alcohol, (2) cannabis, mari*uana, (3) co-use, concurrent use, (4) substitut*, and (5) complement*. Articles were eligible for this review if they examined both cannabis and alcohol use in the same human sample, or if they directly referenced substitution or complementary hypotheses of alcohol and cannabis co-use. In addition to results of these searches, citations were identified within articles as relevant. Finally, colleagues and experts in this area were contacted to inquire about relevant work that was under review or in press.

Results

Search results yielded more than 650 articles, with 95 articles meeting inclusion criteria. The results of this search are organized based on sample composition—clinical or treatment-seeking or -engaged and non–treatment-seeking samples—as motivation for substance use and several other clinical characteristics have been shown to differ between these two subgroups.[12,13] Following this, the review discusses potential mechanisms of the effects of co-use, including pharmacological and behavioral effects of combined alcohol and cannabis use, patterns of co-use, individual differences and user characteristics that may impact co-use, and neurobiological systems that may play a role.

Sample Composition

Treatment-seeking or -engaged samples

In addition to the prospective association between cannabis use and development and persistence of alcohol use disorder (AUD),[14,15] there is evidence that cannabis use may be detrimental to AUD treatment. For instance, cannabis use after discharge from inpatient AUD treatment has been associated with resumed alcohol use.[16] In another study of individuals with AUD recruited during a randomized controlled trial for chronic disease management, cannabis use at study entry was prospectively associated with reduced odds of abstinence from alcohol 1 year post-treatment.[17] Secondary analyses of the Combined Pharmacotherapies and Behavioral Interventions (COMBINE) Study data were conducted to examine the effects of cannabis use on drinking and on alcohol-related consequences 1 year posttreatment.[18,19] In support of the complementary hypothesis, any cannabis use relative to nonuse during treatment was associated with fewer abstinent days at the end of treatment. However, a nuanced association was uncovered such that those who used cannabis once or twice per month had significantly fewer alcohol-abstinent days after treatment. Contrary to the complementary hypothesis, those who used cannabis more frequently (more than twice per month) did not report fewer days abstinent from alcohol. Similarly, those who used cannabis very infrequently (less than once per month) also did not differ from abstainers in terms of their alcohol treatment outcomes. More frequent cannabis use during AUD treatment in Project COMBINE also was associated with increased alcohol-related physical consequences 1 year after treatment.[19] Similarly, another secondary analysis of the U.S. National Alcohol Survey data from the general population subsamples of individuals previously treated for AUD showed that mid-level use (use of cannabis more than monthly but less than weekly) was associated with drinking more frequently, having more drinks per drinking occasion, and being more likely to experience alcohol-related harms relative to abstainers.[20] Together, these studies suggest that cannabis use may be complementary to alcohol use among individuals who have received treatment for AUD, although perhaps only at certain frequencies of use.[18,20]

Conversely, there is some evidence of complementarity among those who drink heavily or those with AUD such that reductions in cannabis use are associated with reduced alcohol use. For example, reduction in cannabis use after treatment for

cannabis use disorder (CUD) was associated with concurrent reduction in alcohol use among those with AUD diagnosis.[21] Concomitant reductions in cannabis use and alcohol use were similarly observed among those who used cannabis, drank heavily (for men, > 14 drinks per week or ≥ 5 drinks per occasion at least once per month over the past 12 months; for women, > 7 drinks per week or ≥ 4 drinks per occasion at least once per month), and were engaged in alcohol interventions.[22,23]

In contrast to these findings suggesting complementary use, patients with AUD report using cannabis specifically to reduce drinking and find it to be an effective substitute for alcohol.[24] In a recent study, cannabis use was assessed during a randomized controlled trial for AUD among enrollees characterized as "heavy drinkers" (defined as 14 drinks/week on average during the past 3 months). Number of cannabis use days (versus days when cannabis was not used) during treatment was associated with reduced alcohol consumption in both frequent and infrequent cannabis users.[25] Conversely, among adolescents undergoing a contingency management intervention for cannabis, an increase in drinking was observed when participants were not using cannabis, whereas a reduction in drinking was observed after cannabis use was reinitiated.[26] This inverse association between cannabis use and alcohol use while in treatment suggests that cannabis in fact may function effectively for some individuals as a substitute for alcohol, but may serve as a complement for others, and thus increasing drinking or exacerbating other alcohol treatment outcomes. Individual differences may be important factors in whether alcohol acts as a substitute for or a complement to cannabis use. These factors and other mechanisms of action are discussed further below.

Non-treatment samples

Studies of co-use associations among individuals not engaged in treatment also help improve our understanding of whether cannabis use leads to increased (i.e., complementary) or decreased (i.e., substitution) drinking. Calendar-assisted interview data in a sample of returning veterans who reported being more likely to drink and drink heavily (> 5 drinks for men/4 drinks for women) on days when they used cannabis suggest a potential complementary pattern.[27] A similar pattern was also found in college students who were interviewed weekly across the first 2 years of college.[28] In another study of college students, complementary consumption was also found in the overall sample.[29] However, students who were more likely to use substances to cope were less likely to use cannabis on evenings when they also drank, suggesting this subpopulation may be more likely to engage in substitution.[29] Studies that specifically examined simultaneous use also found that simultaneous use in non–treatment-seeking users was associated with a heavier quantity of drinking than concurrent use (i.e., using both substances but not at the same time)[6,10,30] or alcohol use alone.[31] This may suggest that the two drugs are more likely to act as complements when they are used in closer proximity to enhance psychoactive effects. As noted above, a more comprehensive review of the impact of simultaneous use on alcohol outcomes is available elsewhere in the topic series.[11]

Research examining the impact of co-use on various substance-related consequences also provides evidence for complementary effects of these two drugs. In college student and young adult samples, significant associations between cannabis use and alcohol-related consequences have been found at the weekly[28] and daily[32,33] levels. Frequent co-use also has been linked to behavioral problems in emergency room samples.[5] Among college students who reported moderate drinking compared with the rest of the sample (measured as recent quantity and frequency of drinking), cannabis use was associated with more alcohol-related problems compared to students who drink at similar levels but do not use cannabis.[34] At the neurocognitive level, co-use during adolescence also has been linked to unique neurocognitive abnormalities compared to single substance use.[35] Longitudinal evidence for complementarity in consequences also exists among adult cannabis users, suggesting that those who continue to use cannabis (compared to abstainers) experience more alcohol-related problems.[36] However, several recent studies suggest that alcohol consumption (i.e., quantity of alcohol consumed at the event level) is a stronger predictor of negative consequences compared to co-use.[31,37-40] The complexity of these findings is discussed in more detail below in the section on patterns of co-use.

In contrast to these studies suggesting complementary patterns, studies that examined the impact of cannabis abstinence on drinking found that abstinence is associated with increased drinking or craving for alcohol in non–treatment-seeking cannabis users.[7,8,41,42] In further support of the substitution hypothesis, findings from a recent within-subjects, placebo-controlled, laboratory study of a non–treatment-seeking sample of persons who used both alcohol and cannabis also suggested that administration of delta-9-tetrahydrocannabinol (THC; 3% and 7%) in smoked cannabis acutely reduced the amount of alcohol consumed on a subsequent drinking task in which participants chose either to drink their preferred alcoholic beverage or receive monetary reinforcement for drinks not consumed.[43] This study also found that THC acutely reduced some dimensions of alcohol craving and alcohol demand measured prior to the alcohol choice task. This direct test of the effect of cannabis on alcohol use in the laboratory suggests that cannabis use prior to the onset of drinking may increase the likelihood that substitution occurs at the event level. Additional consideration of the importance of the order in which substances are consumed and patterns of use is discussed below. Interestingly, in surveys of consumers of medical cannabis, respondents directly endorsed use of cannabis as a substitute for alcohol.[24,44-46] Although it is not clear whether these findings translate to long-term longitudinal research in drinking, the use of medical cannabis as a substitute for alcohol is an important area for future longitudinal research in both treatment-seeking and non–treatment-seeking populations. A more nuanced discussion of medical (versus recreational) cannabis use in relation to alcohol use follows below.

Epidemiology and policy research

Initial evidence for substitution comes from epidemiology and policy-level research. In 2014, Anderson and Rees predicted that cannabis legalization would lead to increased cannabis use, reduced alcohol use, and reduced social harms associated with alcohol.[47] In addition, there has been evidence suggesting increased (almost double) emergency department visits involving alcohol and cannabis exposure among youth.[48,49] However, a recent review of policy literature suggests that medical cannabis laws and other cannabis-related policies resulted in reduction in alcohol sales and alcohol-related fatalities after legalization of cannabis (i.e., substitution), with fewer studies supporting complementary effects or neutral or inconclusive evidence.[9] Of note, examination of complementary versus substitution evidence at a policy level requires a more thorough evaluation of specific laws and is beyond the scope of this review. A more comprehensive review of policy-level work is undertaken elsewhere in this topic series.[50]

Summary

Evidence exists for both substitution and complementary effects across treatment-engaged and community populations of people who co-use alcohol and cannabis; however, individual differences other than treatment engagement (e.g., frequency of use) may contribute to mixed findings within and between these groups. It should be noted that some studies suggest neither substitution nor complementary effects. For example, studies found no change in drinking for samples who are engaged in CUD treatment[51,52] and for a non–treatment-seeking sample that had reduced or abstained from cannabis use after having used it daily.[53] Studies that examine nuanced associations are reviewed in the next section on mechanisms that may aid in understanding the effects of cannabis on alcohol outcomes.

Mechanisms of the Effects of Co-Use

Pharmacological and behavioral effects of co-use

Cannabis has been shown to enhance the positive (i.e., pleasurable) effects of alcohol, increase subjective intoxication, and increase blood alcohol levels at various doses.[54-56] For example, smoking low-potency cannabis (2.5% THC) paired with consumption of a low alcohol dose (0.35 g/kg) increased the number of positive subjective effects endorsed, as well as their duration, and led to higher plasma levels of THC (compared to placebo). In contrast, THC of similar potency paired with a higher dose of alcohol (0.7 g/kg) dampened the increase in plasma ethanol levels and reduced the number and duration of positive subjective effects, even though THC plasma levels were higher than for any other combination of THC potency and alcohol dose.[54,55] Collectively, these findings suggest that the combination of cannabis and alcohol may be more reinforcing at lower alcohol doses than at higher doses. The few human laboratory studies examining the coadministration of alcohol and cannabis also highlight the synergistic effects of the two drugs (i.e., pharmacokinetic interactions).[54-57] In other words, there is consistent evidence that the coadministration of alcohol and cannabis results in increased impairment on a number of behavioral and neurocognitive measures compared to single use of either substance.[58-64] For example, in the presence of alcohol, significantly higher levels of blood THC and 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC, the main active metabolite of THC) are detected, which may explicate increased impairment typically observed following cannabis and alcohol coadministration.[56] However, these studies present limited data regarding the acute influence of cannabis on motivation to consume alcohol. To date, the only study that has directly tested acute effects of cannabis (7% and 3% THC) on alcohol consumption found that smoked cannabis, relative to placebo, acutely reduced the amount of alcohol consumed on a subsequent drinking task.[43] This finding is in line with another laboratory study examining the effects of combined cannabis and alcohol on craving—THC (2.5 mg) with a low dose of alcohol (0.2 g/kg) was actually found to attenuate self-reported "want more drug" (compared to alcohol alone) in a sample of healthy adult volunteers.[57]

Similar to laboratory findings, persons who report regular use of alcohol and cannabis in the natural environment also report an increase in the intoxicating effects of cannabis on days when alcohol is also used.[65] Reports of young adults who engage in simultaneous use suggest that they endorse "cross-fading" motives for using the substances (i.e., using the substances together to achieve increased intoxication).[66] However, what is less clear is whether this increased intoxication consistently motivates increased use in the moment, or whether there are individual differences that account for this association.

Patterns of co-use

The order in which substances are used (i.e., cannabis or alcohol first) has been shown to predict alcohol consumption at the daily level in a sample of college students who use alcohol and cannabis. Specifically, students reported drinking less on days when they reported using cannabis first.[39] This work also controlled for between-person differences, suggesting this effect of order cannot be attributed to person-level differences in cannabis use. However, order of substance use did not predict alcohol-related consequences. This work—in addition to that conducted by others[31,37,38,40]—suggests there are clinically meaningful distinctions in the prediction of alcohol use versus alcohol-related problems. Even when cannabis effects are accounted for in the statistical models, alcohol quantity appears to largely drive the association between co-use (or simultaneous use) and alcohol-related consequences. In other words, the number of drinks consumed in a day or during a drinking event seems to be a more robust predictor of consequences experienced than order of substance use or co-use versus alcohol use only. However, given that prior work has suggested

that co-use may lead to increased alcohol consumption, the association between co-use and consequences may be indirectly driven by number of drinks consumed. Together, these studies underscore the importance of considering all aspects of co-use patterns, including which substance was initiated first in a co-use event, the amount of each drug used, mode or formulation, and perhaps the duration of each use occasion.

Context of co-use is another characteristic that may impact alcohol-related outcomes. A large body of literature has examined the influence of sociocontextual factors on substance use generally,[67] but significantly less work has focused on co-use of alcohol and cannabis specifically. However, recent work has found that social context (i.e., being with others versus being alone) predicts co-use compared to single substance use in both adolescent and young adult samples.[37,68,69] Location of use also predicts co-use over single substance use, in that simultaneous use is more likely to occur at a friend's place, at a party, and when people are around.[69] More work is needed to examine how complex contextual (e.g., social, location, situational) factors interact to predict co-use in more diverse samples, and in treatment-seeking samples, as this may help to inform intervention and prevention efforts.

Individual differences and user characteristics

Between-person differences (i.e., user characteristics) are essential to consider as moderators of the impact of co-use or cannabis use on alcohol-related outcomes. One particularly prominent user characteristic is the use of cannabis for medical or recreational reasons. The 2017 National Academy of Sciences review reported beneficial effects of cannabinoids for several medical conditions, including chronic pain, nausea, and muscle spasms.[70] Aside from evidence for symptom relief in medical conditions such as neuropathic pain and multiple sclerosis, evidence regarding therapeutic effects of cannabis for many other conditions remains elusive. Despite this insufficient evidence, the majority of states have legalized cannabis for medical use, and a number of individuals report using cannabis for medical purposes or assume cannabis has potential health benefits for a variety of physical and mental health conditions.

Although the distinction between "medical" and "recreational" use is likely a false dichotomy, particularly at the between-person level,[71] as individuals may use cannabis for a variety of reasons, several studies have sought to understand how traditional "medical" uses of cannabis may have a unique impact on alcohol-related outcomes. A recent review of the topic found that studies that specifically recruited "medical users" tended to support the substitution hypothesis.[9] For example, in a study of patients using cannabis for medical reasons in California, 40% of the sample directly reported using cannabis as a substitute for alcohol.[24] This intentional substitution also has been reported among veterans who endorsed using cannabis for medicinal reasons[44] and patients registered to use medical cannabis in Canada.[45,46] In line with this, studies that compare "medicinal" users to nonmedical or "recreational" users find that those who use medical cannabis drink less.[72-75] For instance, in studies of returning veterans, less alcohol was consumed among those who reported having used cannabis for medical reasons as compared to those who reported only recreational cannabis use.[76] Specifically, those who used medical cannabis reported consuming less alcohol on days when cannabis was used, but those who used recreational cannabis reported drinking more on cannabis use days. Further, veterans using cannabis for medical reasons who directly endorsed alcohol substitution motives for cannabis use were more likely to drink less on cannabis use days.[44] In another survey on co-use, those using cannabis to treat a medical condition reported drinking less often and having fewer drinks per drinking occasion compared to those who endorsed cannabis use for reasons other than a medical condition.[77] Finally, a large population-based study examined whether having a medical cannabis recommendation from a practitioner had any effect on alcohol consumption among participants who used both alcohol and cannabis. Those with a medical cannabis recommendation showed lower quantity and frequency of alcohol consumption and had lower scores (i.e., alcohol-related problems) on the Alcohol Use Disorders Identification Test (AUDIT) compared to cannabis users without a medical recommendation.[78] Together, this work suggests medical use of cannabis, although difficult to define and not likely to be easily distinguished from "recreational" use,[71] may be associated with less alcohol use and fewer alcohol-related problems.

Age may be another user characteristic that impacts whether cannabis acts as a substitute for or a complement to alcohol and should be considered given recent increases in cannabis use among specific age groups, such as young adults and adults over age 50.[79] Very little work has examined the role of age on co-use. One study examined the impact of medical cannabis laws by age (comparing adolescents to young adults) and found increases in binge drinking in states with medical cannabis laws, but only among those age 21 and older, not among those ages 12 to 20.[80] Although this study is preliminary and correlational in nature, the researchers also found that cannabis use increased in this group after legalization, suggesting that young adults may be more susceptible than adolescents to the complementary effects of cannabis on alcohol use. An alternative consideration may be that adolescents in most states are unable to obtain a medical cannabis card until age 18, which complicates the ability to disentangle potential complementary effects from age-related restrictions to accessing cannabis. In line with this, the review by Risso and colleagues also found that, overall, studies of young adults were more likely to show complementary patterns of consumption (versus substitution patterns).[9] Although preliminary work has begun to examine the potential role of age on co-use, future research should continue to investigate the effects of cannabis and varying cannabinoid compositions on alcohol consumption and patterns of use among diverse age samples.

Several recent investigations have assessed the influence of sex as a moderator of alcohol and cannabis co-use and have reported mixed findings. Much work suggests that the prevalence of co-use is substantially higher among males than females.[6,30,81,82] Purcell and colleagues found that males were more likely to co-use alcohol, tobacco, and cannabis, but females were less likely to use all three substances.[83] However, sex differences were no longer significant when controlling for socioeconomic status; overall, males and females were equally likely to co-use these substances. Roche and colleagues studied event-level reports of alcohol, tobacco, and cannabis use occurring on the same day among individuals endorsing alcohol use.[84] Sex was found to moderate certain patterns of co- or tri-substance use; namely, the association between alcohol and cannabis co-use was greater in males. Moreover, there was an additive effect of co-use of alcohol with tobacco and of cannabis with tobacco on odds of same-day tri-use of tobacco, cannabis, and alcohol, and this effect was more robust in females. Wright and colleagues conducted a laboratory drug administration study to assess for the presence of sex differences in the acute pharmacological effects of alcohol and cannabis co-use.[85] Alcohol and cannabis were administered concurrently in the laboratory using fixed-dose (target, .08% blood alcohol concentration measured through breath) and ad libitum (12.5% THC cannabis) procedures. When alcohol and cannabis were co-administered, females smoked less cannabis as compared to males. Despite this, there were no effects of sex on blood THC concentration, blood pressure, self-reported subjective drug effects, or cognitive assessments. Thus, females were found to experience similar pharmacological and subjective effects of co-use as males, despite differential titration of cannabis in the ad libitum paradigm. Another laboratory drug administration study, conducted by Venegas and colleagues, suggested that administration of alcohol increased craving for cannabis in males but not females;[86] thus, craving may be a mechanism by which alcohol increases risk of co-use in males specifically. Collectively, this research on the influence of sex on co-use suggests that sex appears to influence patterns of co-use, drug self-administration, and craving. Subsequent work should continue to probe the impact of sex on co-use, as well as the impact of gender, for which there is a paucity of work.

Finally, diagnostic status—or the degree of problematic alcohol or cannabis use—is an essential factor to consider when understanding the impact of cannabis use on alcohol consumption and related problems. In particular, in a recent study of veterans who used both alcohol and cannabis, a calendar-assisted data collection method indicated that daily cannabis use was associated with more alcohol consumption among individuals with AUD or both AUD and CUD, but not those with CUD alone. In fact, those with CUD reported drinking less on cannabis use days compared to non-cannabis use days.[27] In another study of college students, higher AUDIT scores before entering college predicted heavier alcohol use on cannabis use days versus nonuse days and more negative alcohol consequences on weeks when more cannabis was used.[28]

The endocannabinoid system and alcohol

The endocannabinoid system regulates both cannabis and alcohol reinforcement, effectively motivating and influencing use of both substances.[87-89] Although it is beyond the scope of this review to discuss the endocannabinoid system in depth (addressed elsewhere in this topic series[90,91]), preclinical models show that cannabinoid receptor agonists and antagonists stimulate and suppress the motivational aspects of alcohol, including its consumption and self-administration.[87] Moreover, long-term exposure to alcohol has been shown to contribute to disruption in endocannabinoid signaling.[58,92] Specifically, chronic alcohol consumption leads to elevated levels of endogenous cannabinoids, ultimately facilitating the downregulation of the cannabinoid receptor type 1,[89,93] a G-coupled receptor that facilitates the psychoactive, intoxicating, and rewarding or positive effects of cannabis.[94,95] This work, taken together, supports the existence of cross-tolerance between alcohol and certain cannabinoids, both exogenous and endogenous. This cross-tolerance could be interpreted to lead to complementary or substitution effects, in that users may seek to use both to increase desired effects of the drug or may effectively substitute one drug for the other in the event they are attempting to reduce their use of a single substance.

Specific cannabinoids and alcohol use and related outcomes

The complexity of cannabinoid composition (e.g., THC-dominant versus cannabidiol [CBD]-dominant cannabis strains), cannabis use patterns (e.g., frequency of use), and formulations (e.g., flower, concentrates, edibles) warrants the investigation of a number of cannabis-specific factors that may moderate the impact of co-use on alcohol outcomes.[96] Prominent among these are cannabinoid composition, potency, and formulation and their pharmacokinetic and pharmacodynamic effects. Although hundreds of cannabinoids in the cannabis plant have been isolated, the two most used and studied are THC (psychoactive) and CBD (nonpsychoactive). In contrast to THC, the nonpsychoactive properties of CBD and its reduced classification as a Schedule I drug in the U.S. Controlled Substances Act have resulted in an influx in the production and consumption of CBD-based products. These products are used as a natural remedy for a wide variety of health issues because of the potential for antioxidant, anti-inflammatory, and analgesic effects.

In addition to this widespread commercial use of CBD, recent preclinical and clinical evidence suggests that CBD may show some efficacy in treatment of a variety of conditions.[97] Among these is the treatment of alcohol-related problems and AUD.[98,99] Preclinical animal models suggest that CBD dampens preference for alcohol, alcohol seeking,[100-102] and alcohol-related functional harms, such as those to the liver and brain.[103-106] There

is significantly less work examining the effects of CBD on alcohol use in humans. In one survey of persons who used cannabis and also drank alcohol, those who reported using products with a higher THC-to-CBD ratio also reported drinking less on drinking days.[77] In a second quasi-experimental study of persons who used cannabis and alcohol, those assigned to purchase and consume CBD products ad libitum, compared to THC or CBD+THC products, reported fewer drinking days and consumed fewer drinks on drinking days.[107] However, there were no differences on either outcome in the groups that were assigned THC compared to THC+CBD, suggesting that CBD does not attenuate the effects of THC on drinking frequency or quantity.[107] This study, although preliminary and not placebo controlled, suggests that use of cannabis products primarily containing CBD is associated with less drinking than use of products containing THC or CBD+THC. This reduction in drinking may be explained by the therapeutic potential of the endocannabinoid system in reducing negative affect among those with AUD or alcohol-related problems.[108] The endocannabinoid system shows promise as a potential target for pharmacological treatments for both AUD and CUD via various mechanisms. Preclinical work suggests that certain ligands that inhibit degradation of endogenous cannabinoids are promising pharmacotherapy targets for both AUD and CUD treatment.[109-112] Cannabinoids also have been shown to reduce the likelihood of development of AUD via their impact on the gastrointestinal and immune system.[113] However, this research is in its infancy, and several ongoing clinical trials seek to better understand the potential of CBD to improve AUD symptoms (i.e., NCT03248167; NCT03252756).

In addition to cannabinoid content, recent work suggests that THC content (i.e., potency) has a significant impact on alcohol-related outcomes. For instance, the use of high-potency products, such as cannabis concentrates, was associated with more alcohol consequences on co-use days among college students who use both alcohol and cannabis.[114] This finding has been replicated in another study based on online surveys of respondents who reported co-use of alcohol and cannabis; respondents were categorized by high- versus low-THC product use. Those categorized as using high-THC products reported drinking more on cannabis use days relative to those who used low-THC products.[77] The significant variability in cannabinoid content and potency in cannabis products, paired with this preliminary evidence that cannabinoid content is associated with alcohol consumption, calls for more controlled research on the impact of various cannabinoids on alcohol use and alcohol-related outcomes (e.g., craving, consequences, high-intensity drinking).

Conclusions and Recommendations for Future Work

Reviews and discussions on cannabis and alcohol co-use thus far have highlighted a mixed set of results regarding whether cannabis acts as a substitute or a complement to alcohol.[7,9,48,115] Although this review is similarly inconclusive given there is evidence for both hypotheses (see Table 1 for summary), several additional moderators and potential mechanisms that help elucidate this complex question and pave the way for future research into this timely topic also have been highlighted (see Figure 1 for summary). Although this review is organized by studies of treatment-seeking or treatment-engaged samples versus those that are not, treatment status itself does not seem to be a clear moderator or indicator of whether cannabis acts as a substitute or complement to alcohol. However, several other mechanisms were identified. For instance, cannabis formulations, including specific cannabinoids (i.e., THC versus CBD), and potency may play a role in whether cannabis acts as a substitute for or a complement to alcohol use, as more compelling preliminary evidence exists that CBD (versus THC) may act as a substitute for alcohol.[98,99,107,113] This evidence is preliminary, however, and additional research and results from ongoing clinical trials are needed to draw definitive conclusions regarding the therapeutic potential of CBD in the treatment of AUD and alcohol-related problems.

In addition, there seems to be a sample-dependent distinction between the impact of cannabis use on alcohol consumption and alcohol-related problems. For instance, evidence among non–treatment-seeking adolescents, young adults, and college students who co-use cannabis and alcohol suggests cannabis may act as a complement to alcohol, given that more drinking is often observed during co-use days or events (and, in particular, simultaneous use events), compared to single substance use.[6,10,28,30,31,116] However, it seems that more frequent or problematic use of a single substance (i.e., alcohol or cannabis) among people who use both also may be indicative of whether cannabis acts as a substitute or complement. For instance, several studies suggest that cannabis use may lead to drinking among those in treatment for AUD (i.e., complementary use).[16-20] However, when those who make heavy use of cannabis abstain from using it, there is consistent evidence for substitution with alcohol.[8,26,41-43] Additionally, participants with CUD, compared to those with AUD, report daily patterns of co-use more consistent with substitution.[27] Motivations for use may be another mechanism by which co-use or cannabis use may impact drinking outcomes. For instance, review of the neurobiological mechanisms suggests that cross-tolerance exists between alcohol and certain cannabinoids. This cross-tolerance may result in increased use among those seeking to experience increased effects from co-use (i.e., "cross-fading"). Alternatively, this cross-tolerance could reinforce substitution motives that might exist, as individuals may

experience desired effects from cannabis and be less likely to reach for alcohol in the moment.

Despite several existing gaps, the current literature may shed additional light on these competing theories of substitution versus complementarity. For instance, preliminary work also suggests that individual differences , such as impulsive personality,[117] may impact drinking rates on co-use days (i.e., less impulsive individuals are more likely to substitute cannabis for alcohol). Additional individual differences that may be factors in whether cannabis acts as a substitute for or a complement to alcohol are important to examine, as both alcohol and cannabis act on the same neural reward pathway; therefore, individual differences in reward sensitivity may interact with co-use to predict unique substitution versus complementary effects. Further, there is a significant dearth of research examining demographic factors—such as sex, race, and ethnicity—that likely play a role in co-use of alcohol and cannabis. For example, evidence from preclinical work suggests that there may be an age-dependent decline in cannabis and alcohol interactions independent from exposure to or level of experience with either substance,[118] suggesting that age may moderate the level of substitution or complementarity one endorses. Additionally, it should be noted that early studies on cannabis occurred within a criminalized environment, which has led to increased stigmatization of cannabis use[119] and, therefore, may reduce the ability of those who use cannabis to effectively use it as a substitute for alcohol. Finally, all studies reviewed examined co-use at a single level of analysis (e.g., laboratory administration studies, self-report daily survey studies). However, complex interactions between individual pharmacokinetic response to substance use and an individual's sociocontextual environment may exist. Mixed-methods studies that cut across these rigorous levels of data collection may help to elucidate how each of these mechanisms (e.g., context of co-use, timing and order of use, cannabinoid formulations, pharmacokinetic interactions, user characteristics) contributes to substitution versus complementary patterns of use. Taken together, these studies highlight the complex nature of cannabis and alcohol co-use and ultimately suggest that internal (e.g., pharmacological) and external (e.g., context) factors interact to yield complementary and substitution effects of alcohol and cannabis that likely shift over time, throughout the day, and potentially in the moment. Further investigation is needed to continue to clarify differences in patterns and context of cannabis use as either a complement to or a substitute for alcohol use at both within- and between-person levels.

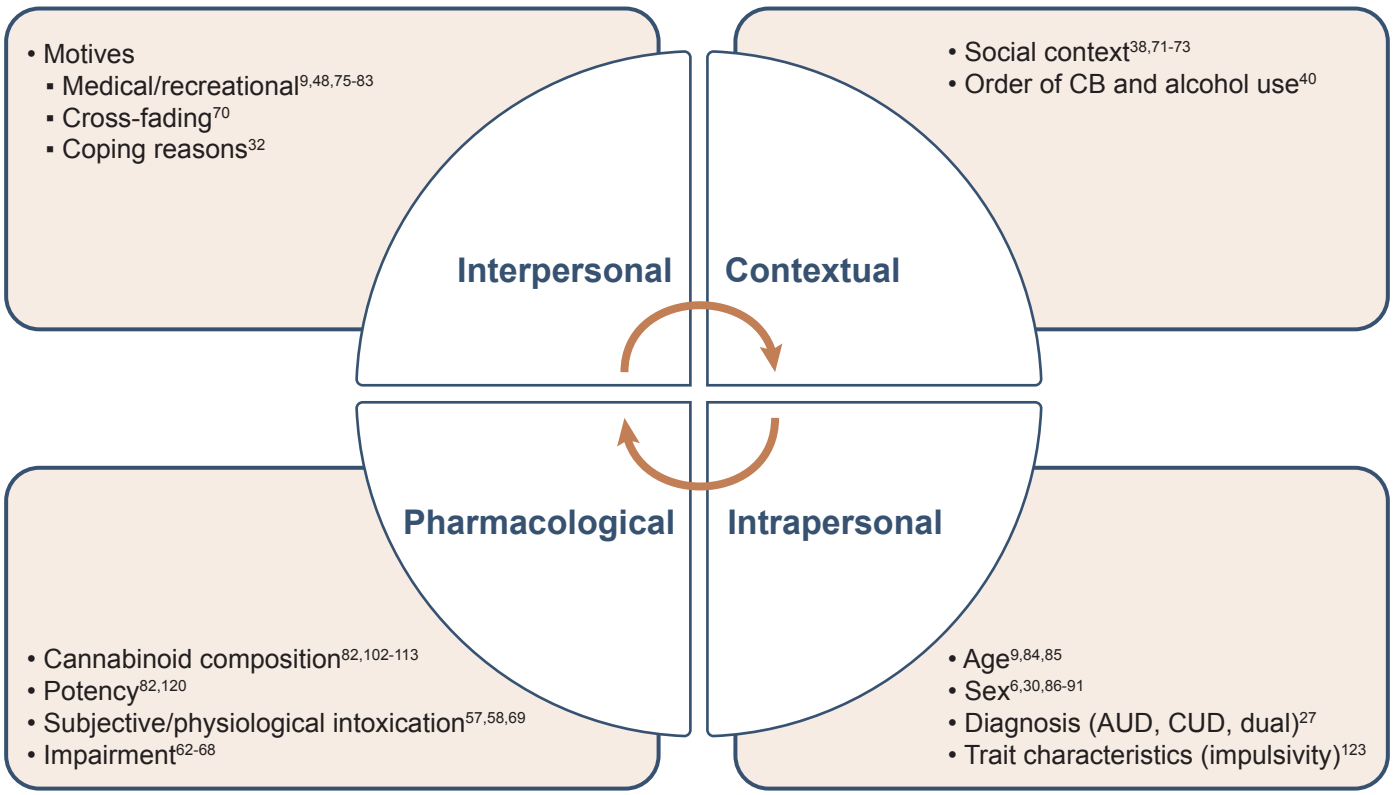

Figure 1. Mechanisms of the effects of cannabis and alcohol co-use. *Note:* AUD, alcohol use disorder; CB, cannabis; CUD, cannabis use disorder.

Table 1. Effects of Substitution or Complementary Use of Cannabis on Use of Alcohol and Alcohol-Related Consequences, by Sample

Substitution	
Clinical or Treatment-Seeking or -Engaged	
Alcohol use	**Alcohol-related consequences**
Self-reported substitution of ALC with CB[24]	
CB use days associated with lower ALC consumption after ALC Tx[25]	
Decreased CB after contingency management Tx for CUD associated with increased ALC use; reinitiated CB associated with decreased ALC use[26]	
Non–Treatment-Seeking	
Alcohol use	**Alcohol-related consequences**
CB abstinence associated with increased ALC use[7,8,41,42]	
THC administration associated with increased ALC use and craving[43]	
Combined ALC and CB associated with lower "want more drug"[57]	
Complementary	
Clinical or Treatment-Seeking or -Engaged	
Alcohol use	**Alcohol-related consequences**
CB use after AUD Tx associated with resumed ALC use[16]	More frequent CB use during AUD Tx associated with increased ALC consequences 1 year after Tx[19]
CB at AUD Tx entry associated with reduced abstinence[17]	CB use predicts AUD[14,15]
Mid-level CB use frequency during/after AUD Tx associated with fewer abstinent days after Tx, higher quantity, and greater frequency[18,20]	
Reduced CB use after CUD Tx associated with reduced ALC use among those with AUD[21]	
Reductions in ALC and CB use among persons in ALC Tx who report heavy drinking and CB use[22,23]	
Non–Treatment-Seeking	
Alcohol use	**Alcohol-related consequences**
Daily CB use associated with more ALC use[27,28]	CB use associated with increased ALC consequences[28,32-34,36]
Simultaneous use associated with more ALC use[6,10,30]	Co-use associated with neurocognitive abnormalities[35]

Note: ALC, alcohol; AUD, alcohol use disorder; CB, cannabis; CUD, cannabis use disorder; Tx, treatment

References

1. Centers for Disease Control and Prevention. Alcohol and Public Health: Alcohol-Related Disease Impact (ARDI) Application. https://nccd.cdc.gov/DPH_ARDI/default/default.aspx.
2. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004;291(10):1238-1245. https://doi.org/10.1001/jama.291.10.1238.
3. Substance Abuse and Mental Health Services Administration. *Key Substance Use and Mental Health Indicators in the United States: Results From the 2018 National Survey on Drug Use and Health*. Rockville, MD: U.S. Department of Health and Human Services; 2019. https://www.samhsa.gov/data/sites/default/files/cbhsq-reports/NSDUHNationalFindingsReport2018/NSDUHNationalFindingsReport2018.pdf.
4. Midanik LT, Tam TW, Weisner C. Concurrent and simultaneous drug and alcohol use: Results of the 2000 National Alcohol Survey. *Drug Alcohol Depend*. 2007;90(1):72-80. https://doi.org/10.1016/j.drugalcdep.2007.02.024.
5. Harrington M, Baird J, Lee C, et al. Identifying subtypes of dual alcohol and marijuana users: A methodological approach using cluster analysis. *Addict Behav*. 2012;37(1):119-123. https://doi.org/10.1016/j.addbeh.2011.07.016.
6. Subbaraman MS, Kerr WC. Simultaneous versus concurrent use of alcohol and cannabis in the National Alcohol Survey. *Alcohol Clin Exp Res*. 2015;39(5):872-879. https://doi.org/10.1111/acer.12698.
7. Subbaraman MS. Substitution and complementarity of alcohol and cannabis: A review of the literature. *Subst Use Misuse*. 2016;51(11):1399-1414. https://doi.org/10.3109/10826084.2016.1170145.
8. Peters EN, Hughes JR. Daily marijuana users with past alcohol problems increase alcohol consumption during marijuana abstinence. *Drug Alcohol Depend*. 2010;106(2-3):111-118. https://doi.org/10.1016/j.drugalcdep.2009.07.027.
9. Risso C, Boniface S, Subbaraman MS, Englund A. Does cannabis complement or substitute alcohol consumption? A systematic review of human and animal studies. *J Psychopharmacol*. 2020;34(9):938-954. https://doi.org/10.1177/0269881120919970.
10. Patrick ME, Kloska DD, Terry-McElrath YM, Lee CM, O'Malley PM, Johnston LD. Patterns of simultaneous and concurrent alcohol and marijuana use among adolescents. *Am J Drug Alcohol Abuse*. 2017;44(4):441-451. https://doi.org/10.1080/00952990.2017.1402335.
11. Lee CM, Calhoun BH, Abdallah DA, et al. Simultaneous alcohol and marijuana use among young adults: A scoping review of prevalence, patterns, psychosocial correlates, and consequences. *Alcohol Res*. In press.
12. Ray LA, Bujarski S, Yardley MM, Roche DJO, Hartwell EE. Differences between treatment-seeking and nontreatment-seeking participants in medication studies for alcoholism: Do they matter? *Am J Drug Alcohol Abuse*. 2017;43(6):703-710. https://doi.org/10.1080/00952990.2017.1312423.
13. Rohn MCH, Lee MR, Kleuter SB, Schwandt ML, Falk DE, Leggio L. Differences between treatment-seeking and nontreatment-seeking alcohol-dependent research participants: An exploratory analysis. *Alcohol Clin Exp Res*. 2017;41(2):414-420. https://doi.org/10.1111/acer.13304.
14. Blanco C, Hasin DS, Wall MM, et al. Cannabis use and risk of psychiatric disorders: Prospective evidence from a US National longitudinal study. *JAMA Psychiatry*. 2016;73(4):388-395. https://doi.org/10.1001/jamapsychiatry.2015.3229.
15. Weinberger AH, Platt J, Goodwin RD. Is cannabis use associated with an increased risk of onset and persistence of alcohol use disorders? A three-year prospective study among adults in the United States. *Drug Alcohol Depend*. 2016;161:363-367. https://doi.org/10.1016/j.drugalcdep.2016.01.014.
16. Aharonovich E, Liu X, Samet S, Nunes E, Waxman R, Hasin D. Postdischarge cannabis use and its relationship to cocaine, alcohol, and heroin use: A prospective study. *Am J Psychiatry*. 2005;162(8):1507-1514. https://doi.org/10.1176/appi.ajp.162.8.1507.
17. Mojarrad M, Samet JH, Cheng DM, Winter MR, Saitz R. Marijuana use and achievement of abstinence from alcohol and other drugs among people with substance dependence: A prospective cohort study. *Drug Alcohol Depend*. 2014;142:91-97. https://doi.org/10.1016/j.drugalcdep.2014.06.006.
18. Subbaraman MS, Metrik J, Patterson D, Swift R. Cannabis use during treatment for alcohol use disorders predicts alcohol treatment outcomes. *Addiction*. 2017;112(4):685-694. https://doi.org/10.1111/add.13693.
19. Subbaraman M, Metrik J, Patterson D, Stout R. Cannabis use during alcohol treatment is associated with alcohol-related problems one-year post-treatment. *Drug Alcohol Depend*. 2018;193:29-34. https://doi.org/10.1016/j.drugalcdep.2018.08.020.
20. Subbaraman MS, Barnett SB, Karriker-Jaffe KJ. Risks associated with mid level cannabis use among people treated for alcohol use disorder. *Alcohol Clin Exp Res*. 2019;43(4):690-694. https://doi.org/10.1111/acer.13973.
21. Dunn HK, Litt MD. Decreased drinking in adults with co-occurring cannabis and alcohol use disorders in a treatment trial for marijuana dependence: Evidence of a secondary benefit? *Addict Behav*. 2019;99:106051. https://doi.org/10.1016/j.addbeh.2019.106051.
22. Magill M, Barnett NP, Apodaca TR, Rohsenow DJ, Monti PM. The role of marijuana use in brief motivational intervention with young adult drinkers treated in an emergency department. *J Stud Alcohol Drugs*. 2009;70(3):409-413. https://doi.org/10.15288/jsad.2009.70.409.
23. Metrik J, Spillane NS, Leventhal AM, Kahler CW. Marijuana use and tobacco smoking cessation among heavy alcohol drinkers. *Drug Alcohol Depend*. 2011;119(3):194-200. https://doi.org/10.1016/j.drugalcdep.2011.06.004.
24. Reiman A. Cannabis as a substitute for alcohol and other drugs. *Harm Reduct J*. 2009;6:35. https://doi.org/10.1186/1477-7517-6-35.
25. Karoly HC, Ross JM, Prince MA, Zabelski AE, Hutchison KE. Effects of cannabis use on alcohol consumption in a sample of treatment-engaged heavy drinkers in Colorado. *Addiction*. 2021;116(9):2529-2537. https://doi.org/10.1111/add.15407.
26. Schuster RM, Potter K, Lamberth E, et al. Alcohol substitution during one month of cannabis abstinence among non-treatment seeking youth. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2021;107:110205. https://doi.org/10.1016/j.pnpbp.2020.110205.
27. Metrik J, Gunn RL, Jackson KM, Sokolovsky AW, Borsari B. Daily patterns of marijuana and alcohol co-use among individuals with alcohol and cannabis use disorders. *Alcohol Clin Exp Res*. 2018;42(6):1096-1104. https://doi.org/10.1111/acer.13639.
28. Gunn RL, Norris AL, Sokolovsky A, Micalizzi L, Jennifer E, Barnett NP. Marijuana use is associated with alcohol use and consequences across the first 2 years of college. *Psychol Addict Behav*. 2018;32(8):885-894. https://doi.org/10.1037/adb0000416.

29. O'Hara RE, Armeli S, Tennen H. Alcohol and cannabis use among college students: Substitutes or complements? *Addict Behav.* 2016;58:1-6. https://doi.org/10.1016/j.addbeh.2016.02.004.
30. Brière FN, Fallu JS, Descheneaux A, Janosz M. Predictors and consequences of simultaneous alcohol and cannabis use in adolescents. *Addict Behav.* 2011;36(7):785-788. https://doi.org/10.1016/j.addbeh.2011.02.012.
31. Lee CM, Patrick ME, Fleming CB, et al. A daily study comparing alcohol-related positive and negative consequences for days with only alcohol use versus days with simultaneous alcohol and marijuana use in a community sample of young adults. *Alcohol Clin Exp Res.* 2020;44(3):689-696. https://doi.org/10.1111/acer.14279.
32. Mallett KA, Turrisi R, Hultgren BA, Sell N, Reavy R, Cleveland M. When alcohol is only part of the problem: An event-level analysis of negative consequences related to alcohol and other substance use. *Psychol Addict Behav.* 2017;31(3):307-314. https://doi.org/10.1037/adb0000260.
33. Linden-Carmichael AN, Van Doren N, Masters LD, Lanza ST. Simultaneous alcohol and marijuana use in daily life: Implications for level of use, subjective intoxication, and positive and negative consequences. *Psychol Addict Behav.* 2020;34(3):447-453. https://doi.org/10.1037/adb0000556.
34. Haas AL, Wickham R, Macia K, Shields M, Macher R, Schulte T. Identifying classes of conjoint alcohol and marijuana use in entering freshmen. *Psychol Addict Behav.* 2015;29(3):620-626. https://doi.org/10.1037/adb0000089.
35. Medina KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF. Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal volume and asymmetry. *Neurotoxicol Teratol.* 2007;29(1):141-152. https://doi.org/10.1016/j.ntt.2006.10.010.
36. Choi NG, DiNitto DM, Marti CN. A longitudinal assessment of change in marijuana use with other substance use problems. *Am J Drug Alcohol Abuse.* 2018;44(6):642-652. https://doi.org/10.1080/00952990.2018.1461879.
37. Lipperman-Kreda S, Gruenewald PJ, Grube JW, Bersamin M. Adolescents, alcohol, and marijuana: Context characteristics and problems associated with simultaneous use. *Drug Alcohol Depend.* 2017;179:55-60. https://doi.org/10.1016/j.drugalcdep.2017.06.023.
38. Gunn RL, Sokolovsky A, Stevens AK, Metrik J, White H, Jackson K. Ordering in alcohol and cannabis co-use: Impact on daily consumption and consequences. *Drug Alcohol Depend.* 2021;218:108339. https://doi.org/10.1016/j.drugalcdep.2020.108339.
39. Lee CM, Patrick ME, Fleming CB, et al. A daily study comparing alcohol-related positive and negative consequences for days with only alcohol use versus days with simultaneous alcohol and marijuana use in a community sample of young adults. *Alcohol Clin Exp Res.* 2020;44(3):689-696. https://doi.org/10.1111/acer.14279.
40. Sokolovsky AW, Gunn RL, Micalizzi L, White HR, Jackson KM. Alcohol and marijuana co-use: Consequences, subjective intoxication, and the operationalization of simultaneous use. *Drug Alcohol Depend.* 2020;212:107986. https://doi.org/10.1016/j.drugalcdep.2020.107986.
41. Copersino M, Boyd S, Tashkin D, et al. Quitting among non-treatment-seeking marijuana users: Reasons and changes in other substance use. *Am J Addict.* 2006;15(4):297-302. https://doi.org/10.1080/10550490600754341.
42. Allsop DJ, Dunlop AJ, Saddler C, Rivas GR, McGregor IS, Copeland J. Changes in cigarette and alcohol use during cannabis abstinence. *Drug Alcohol Depend.* 2014;138:54-60. https://doi.org/10.1016/j.drugalcdep.2014.01.022.
43. Metrik J, Aston ER, Gunn RL, MacKillop J, Swift R, Kahler CW. Marijuana's effects on alcohol craving and consumption in a laboratory study. Paper presented at the 42nd Annual Scientific Meeting of the Research Society on Alcoholism Conference. *Alcohol Clin Exp Res.* 2019:Suppl-S1, P286A.
44. Gunn R, Jackson K, Borsari B, Metrik J. A longitudinal examination of daily patterns of cannabis and alcohol co-use among medicinal and recreational veteran cannabis users. *Drug Alcohol Depend.* 2019;205:107661. https://doi.org/10.1016/j.drugalcdep.2019.107661.
45. Lucas P, Walsh Z. Medical cannabis access, use, and substitution for prescription opioids and other substances: A survey of authorized medical cannabis patients. *Int J Drug Policy.* 2017;42:30-35. https://doi.org/10.1016/j.drugpo.2017.01.011.
46. Lucas P, Baron EP, Jikomes N. Medical cannabis patterns of use and substitution for opioids & other pharmaceutical drugs, alcohol, tobacco, and illicit substances; results from a cross-sectional survey of authorized patients. *Harm Reduct J.* 2019;16(1):9. https://doi.org/10.1186/s12954-019-0278-6.
47. Anderson DM, Rees DI. The legalization of recreational marijuana: How likely is the worst-case scenario? *J Policy Anal Manag.* 2014;33(1):221-232. https://doi.org/10.1002/pam.21727.
48. Pacula RL, Sevigny EL. Natural experiments in a complex and dynamic environment: The need for measured assessment of the evidence. *J Policy Anal Manag.* 2014;33(1):232-235. https://doi.org/10.1002/pam.21730.
49. National Institute on Drug Abuse, Institute for Social Research at University of Michigan. Drug Abuse Warning Network (DAWN), 2010 (ICPSR34083). Ann Arbor, MI: Inter-university Consortium for Political and Social Research (ICPSR); 2010. https://doi.org/10.3886/ICPSR34083.v2.
50. Pacula RL, Smart R, Lira MC, Pessar SC, Blanchette JG, Naimi TS. Relationships of cannabis policy liberalization with alcohol use and co-use with cannabis: A narrative review. *Alcohol Res.* In press.
51. Kadden RM, Litt MD, Kabela-Cormier E, Petry NM. Increased drinking in a trial of treatments for marijuana dependence: Substance substitution? *Drug Alcohol Depend.* 2009;105(1-2):168-171. https://doi.org/10.1016/j.drugalcdep.2009.05.024.
52. Babor TF, Marijuana Treatment Project Research Group. Brief treatments for cannabis dependence: Findings from a randomized multisite trial. *J Consult Clin Psychol.* 2004;72(3):455-466. https://doi.org/10.1037/0022-006x.72.3.455.
53. Hughes JR, Peters EN, Callas PW, Budney AJ, Livingston AE. Attempts to stop or reduce marijuana use in non-treatment seekers. *Drug Alcohol Depend.* 2008;97(1-2):180-184. https://doi.org/10.1016/j.drugalcdep.2008.03.031.
54. Lukas SE, Orozco S. Ethanol increases plasma Δ^9-tetrahydrocannabinol (THC) levels and subjective effects after marihuana smoking in human volunteers. *Drug Alcohol Depend.* 2001;64(2):143-149. https://doi.org/10.1016/s0376-8716(01)00118-1.
55. Lukas S, Benedikt R, Mendelson JH, Kouri E, Sholar M, Amass L. Marihuana attenuates the rise in plasma ethanol levels in human subjects. *Neuropsychopharmacology.* 1992;7(1):77-81.
56. Hartman RL, Brown TL, Milavetz G, et al. Controlled cannabis vaporizer administration: Blood and plasma cannabinoids with and without alcohol. *Clin Chem.* 2015;61(6):850-869. https://doi.org/10.1373/clinchem.2015.238287.
57. Ballard ME, De Wit H. Combined effects of acute, very-low-dose ethanol and delta(9)-tetrahydrocannabinol in healthy human volunteers. *Pharmacol Biochem Behav.* 2011;97(4):627-631. https://doi.org/10.1016/j.pbb.2010.11.013.

58. Pava MJ, Woodward JJ. A review of the interactions between alcohol and the endocannabinoid system: Implications for alcohol dependence and future directions for research. *Alcohol.* 2012;46(3):185-204. https://doi.org/10.1016/j.alcohol.2012.01.002.
59. Perez-Reyes M, Hicks RE, Bumberry J, Jeffcoat ARJ, Cook CE. Interaction between marihuana and ethanol: Effects on psychomotor performance. *Alcohol Clin Exp Res.* 1988;12(2):268-276. https://doi.org/10.1111/j.1530-0277.1988.tb00193.x.
60. Bramness JG, Khiabani HZ, Mørland J. Impairment due to cannabis and ethanol: Clinical signs and additive effects. *Addiction.* 2010;105(6):1080-1087. https://doi.org/10.1111/j.1360-0443.2010.02911.x.
61. Ramaekers JG, Robbe HWJ, O'Hanlon JF. Marijuana, alcohol and actual driving performance. *Hum Psychopharmacol.* 2000;15(7):551-558. https://doi.org/10.1002/1099-1077(200010)15:7%3C551::aid-hup236%3E3.0.co;2-p.
62. Chait LD, Perry JL. Factors influencing self-administration of, and subjective response to, placebo marijuana. *Behav Pharmacol.* 1992;3(6):545-552.
63. Heishman SJ, Arasteh K, Stitzer ML. Comparative effects of alcohol and marijuana on mood, memory, and performance. *Pharmacol Biochem Behav.* 1997;58(1):93-101. https://doi.org/10.1016/s0091-3057(96)00456-x.
64. Ramaekers JG, Theunissen EL, De Brouwer M, Toennes SW, Moeller MR, Kauert G. Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. *Psychopharmacology (Berl).* 2011;214(2):391-401. https://doi.org/10.1007/s00213-010-2042-1.
65. Hughes JR, Fingar JR, Budney AJ, Naud S, Helzer JE, Callas PW. Marijuana use and intoxication among daily users: An intensive longitudinal study. *Addict Behav.* 2014;39(10):1464-1470. https://doi.org/10.1016/j.addbeh.2014.05.024.
66. Patrick ME, Fairlie AM, Lee CM. Motives for simultaneous alcohol and marijuana use among young adults. *Addict Behav.* 2018;76:363-369. https://doi.org/10.1016/j.addbeh.2017.08.027.
67. Freisthler B, Lipperman-Kreda S, Bersamin M, Gruenewald PJ. Tracking the when, where, and with whom of alcohol use: Integrating ecological momentary assessment and geospatial data to examine risk for alcohol-related problems. *Alcohol Res.* 2014;36(1):29-38.
68. Linden-Carmichael AN, Allen HK, Lanza ST. The socio-environmental context of simultaneous alcohol and marijuana use among young adults: Examining day-level associations. *Drug Alcohol Rev.* 2021;40(4):647-657. https://doi.org/10.1111/dar.13213.
69. Gunn RL, Sokolovsky AW, Stevens AK, et al. Contextual influences on simultaneous alcohol and cannabis use in a predominately white sample of college students. *Psychol Addict Behav.* 2021;35(6):691-697. https://doi.org/10.1037/adb0000739.
70. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Population Health and Public Health Practice; Committee on the Health Effects of Marijuana: An Evidence Review and Research Agenda. *The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research.* Washington, DC: National Academies Press; 2017.
71. Budney AJ. Teen reports of cannabis for medical reasons—what does that mean? *J Adolesc Heal.* 2021;68(1):9-10. https://doi.org/10.1016/j.jadohealth.2020.09.044.
72. Lin LA, Ilgen MA, Jannausch M, Bohnert KM. Comparing adults who use cannabis medically with those who use recreationally: Results from a national sample. *Addict Behav.* 2016;61:99-103. https://doi.org/10.1016/j.addbeh.2016.05.015.
73. Loflin M, Earleywine M, Bonn-Miller M. Medicinal versus recreational cannabis use: Patterns of cannabis use, alcohol use, and cued-arousal among veterans who screen positive for PTSD. *Addict Behav.* 2017;68:18-23. https://doi.org/10.1016/j.addbeh.2017.01.008.
74. Roy-Byrne P, Maynard C, Bumgardner K, et al. Are medical marijuana users different from recreational users? The view from primary care. *Am J Addict.* 2015;24(7):599-606. https://doi.org/10.1111/ajad.12270.
75. Woodruff SI, Shillington AM. Sociodemographic and drug use severity differences between medical marijuana users and non-medical users visiting the emergency department. *Am J Addict.* 2016;25(5):385-391. https://doi.org/10.1111/ajad.12401.
76. Metrik J, Bassett S, Aston ER, Jackson K, Borsari B. Medicinal versus recreational cannabis use among returning veterans. *Transl Issues Psychol Sci.* 2018;4(1):6-20. https://doi.org/10.1037/tps0000133.
77. Karoly HC, Mueller RL, Andrade CC, Hutchison KE. Investigating relationships between alcohol and cannabis use in an online survey of cannabis users: A focus on cannabinoid content and cannabis for medical purposes. *Front Psychiatry.* 2020;11:613243. https://doi.org/10.3389/fpsyt.2020.613243.
78. Subbaraman M, Kerr WC. Alcohol use and risk of related problems among cannabis users is lower among those with medical cannabis recommendations, though not due to health. *J Stud Alcohol Drugs.* 2018;79(6):935-942. https://doi.org/10.15288/jsad.2018.79.935.
79. Han BH, Sherman S, Mauro PM, Martins SS, Rotenberg J, Palamar JJ. Demographic trends among older cannabis users in the United States, 2006-13. *Addiction.* 2017;112(3):516-525. https://doi.org/10.1111/add.13670.
80. Wen H, Hockenberry JM, Cummings JR. The effect of medical marijuana laws on adolescent and adult use of marijuana, alcohol, and other substances. *J Health Econ.* 2015;42:64-80. https://doi.org/10.1016/j.jhealeco.2015.03.007.
81. Höhne B, Pabst A, Hannemann TV, Kraus L. Patterns of concurrent alcohol, tobacco, and cannabis use in Germany: Prevalence and correlates. *Drugs Educ Prev Policy.* 2014;21(2):102-109. https://doi.org/10.3109/09687637.2013.812614.
82. Patrick ME, Terry-McElrath YM, Lee CM, Schulenberg JE. Simultaneous alcohol and marijuana use among underage young adults in the United States. *Addict Behav.* 2019;88:77-81. https://doi.org/10.1016/j.addbeh.2018.08.015.
83. Purcell JB, Orihuela CA, Elliott MN, Tortolero Emery S, Schuster MA, Mrug S. Examining sex and racial/ethnic differences in co-use of alcohol, cannabis, and cigarettes in a community sample of adolescents. *Subst Use Misuse.* 2021;56(1):101-110. https://doi.org/10.1080/10826084.2020.1843056.
84. Roche DJO, Bujarski S, Green R, Hartwell EE, Leventhal AM, Ray LA. Alcohol, tobacco, and marijuana consumption is associated with increased odds of same-day substance co- and tri-use. *Drug Alcohol Depend.* 2019;200:40-49. https://doi.org/10.1016/j.drugalcdep.2019.02.035.
85. Wright M, Wickens CM, Di Ciano P, et al. Sex differences in the acute pharmacological and subjective effects of smoked cannabis combined with alcohol in young adults. *Psychol Addict Behav.* 2021;35(5):536-552. https://doi.org/10.1037/adb0000749.
86. Venegas A, Meredith LR, Green RJ, Cooper ZD, Ray LA. Sex-dependent effects of alcohol administration on the urge to use cannabis. *Exp Clin Psychopharmacol.* 2020;10.1037/pha0000409. https://doi.org/10.1037/pha0000409.

87. Colombo G, Serra S, Vacca G, Carai MAM, Gessa GL. Endocannabinoid system and alcohol addiction: Pharmacological studies. *Pharmacol Biochem Behav*. 2005;81(2):369-380. https://doi.org/10.1016/j.pbb.2005.01.022.
88. López-Moreno JA, Echeverry-Alzate V, Bü KM. The genetic basis of the endocannabinoid system and drug addiction in humans. *J Psychopharmacol*. 2012;26(1):133-143. https://doi.org/10.1177/0269881111416689.
89. Hungund BL, Basalingappa BS. Role of endocannabinoids and cannabinoid CB1 receptors in alcohol-related behaviors. *Ann N Y Acad Sci*. 2004;1025(1):515-527. https://doi.org/10.1196/annals.1316.064.
90. Serrano A, Natividad LA. Alcohol–endocannabinoid interactions in addiction behavior. *Alcohol Res*. In press.
91. Wolfe SA, Vozella V, Roberto M. The synaptic interactions of alcohol and the endogenous cannabinoid system. *Alcohol Res*. 2022;42(1). https://arcr.niaaa.nih.gov/niaaa-50th-anniversary-festschrift/synaptic-interactions-alcohol-and-endogenous-cannabinoid-system.
92. Pavón FJ, Serrano A, Stouffer DG, et al. Ethanol-induced alterations in endocannabinoids and relevant neurotransmitters in the nucleus accumbens of fatty acid amide hydrolase knockout mice. *Addict Biol*. 2019;24(6):1204-1215. https://doi.org/10.1111/adb.12695.
93. Malinen H, Hyytiä P. Ethanol self-administration is regulated by CB1 receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. *Alcohol Clin Exp Res*. 2008;32(11):1976-1983. https://doi.org/10.1111/j.1530-0277.2008.00786.x.
94. Ledent C, Valverde O, Cossu G, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. *Science*. 1999;283(5400):401-404. https://doi.org/10.1126/science.283.5400.401.
95. Zhang P-W, Ishiguro H, T Ohtsuki, et al. Human cannabinoid receptor 1: 5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. *Mol Psychiatry*. 2004;9(10):916-931. https://doi.org/10.1038/sj.mp.4001560.
96. Metrik J. Cannabis and Alcohol Co-Use and Comorbidity [white paper]. Austin, TX, Research Society on Alcoholism, 2020. https://www.xcdsystem.com/rsoa/files/Cannabis%20and%20Alcohol%20Co-Use.pdf.
97. Wheeler M, Merten JW, Gordon BT, Hamadi H. CBD (cannabidiol) product attitudes, knowledge, and use among young adults. *Subst Use Misuse*. 2020;55(7):1138-1145. https://doi.org/10.1080/10826084.2020.1729201.
98. Turna J, Syan SK, Frey BN, et al. Cannabidiol as a novel candidate alcohol use disorder pharmacotherapy: A systematic review. *Alcohol Clin Exp Res*. 2019;43(4):550-563. https://doi.org/10.1111/acer.13964.
99. Nona CN, Hendershot CS, Le Foll B. Effects of cannabidiol on alcohol-related outcomes: A review of preclinical and human research. *Exp Clin Psychopharmacol*. 2019;27(4):359-369. https://doi.org/10.1037/pha0000272.
100. Gonzalez-Cuevas G, Martin-Fardon R, Kerr TM, et al. Unique treatment potential of cannabidiol for the prevention of relapse to drug use: Preclinical proof of principle. *Neuropsychopharmacology*. 2018;43(10):2036-2045. https://doi.org/10.1038/s41386-018-0050-8.
101. Viudez-Martínez A, García-Gutiérrez MS, Navarrón CM, et al. Cannabidiol reduces ethanol consumption, motivation and relapse in mice. *Addict Biol*. 2018;23(1):154-164. https://doi.org/10.1111/adb.12495.
102. Maccioni P, Bratzu J, Carai MAM, Colombo G, Gessa GL. Reducing effect of cannabidiol on alcohol self-administration in Sardinian alcohol-preferring rats. *Cannabis Cannabinoid Res*. 2021. https://doi.org/10.1089/can.2020.0132.
103. Wang Y, Mukhopadhyay P, Cao Z, et al. Cannabidiol attenuates alcohol-induced liver steatosis, metabolic dysregulation, inflammation and neutrophil-mediated injury. *Sci Rep*. 2017;7(1):12064. https://doi.org/10.1038/s41598-017-10924-8.
104. Yang L, Rozenfeld R, Wu D, Devi LA, Zhang Z, Cederbaum A. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. *Free Radic Biol Med*. 2014;68:260-267. https://doi.org/10.1016/j.freeradbiomed.2013.12.026.
105. Hamelink C, Hampson A, Wink DA, Eiden LE, Eskay RL. Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. *J Pharmacol Exp Ther*. 2005;314(2):780-788. https://doi.org/10.1124/jpet.105.085779.
106. Avraham Y, Grigoriadis N, Poutahidis T, et al. Cannabidiol improves brain and liver function in a fulminant hepatic failure-induced model of hepatic encephalopathy in mice. *Br J Pharmacol*. 2011;162(7):1650-1658. https://doi.org/10.1111/j.1476-5381.2010.01179.x.
107. Karoly HC, Mueller RL, Andrade CC, Hutchison KE. THC and CBD effects on alcohol use among alcohol and cannabis co-users. *Psychol Addict Behav*. 2021;35(6):749-759. https://doi.org/10.1037/adb0000706.
108. Bedse G, Centanni SW, Winder DG, Patel S. Endocannabinoid signaling in the central amygdala and bed nucleus of the stria terminalis: Implications for the pathophysiology and treatment of alcohol use disorder. *Alcohol Clin Exp Res*. 2019;43(10):2014-2027. https://doi.org/10.1111/acer.14159.
109. Zhou Y, Schwartz BI, Giza J, Gross SS, Lee FS, Kreek MJ. Blockade of alcohol escalation and "relapse" drinking by pharmacological FAAH inhibition in male and female C57BL/6J mice. *Psychopharmacology (Berl)*. 2017;234(19):2955-2970. https://doi.org/10.1007/s00213-017-4691-9.
110. Blednov YA, Cravatt BF, Boehm II SL, Walker D, Harris RA. Role of endocannabinoids in alcohol consumption and intoxication: Studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology*. 2007;32(7):1570-1582. https://doi.org/10.1038/sj.npp.1301274.
111. Cippitelli A, Cannella N, Braconi S, et al. Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology (Berl)*. 2008;198(4):449-460. https://doi.org/10.1007/s00213-008-1104-0.
112. D'Souza DC, Cortes-Briones J, Creatura G, et al. Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: A double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. *Lancet Psychiatry*. 2019;6(1):35-45. https://doi.org/10.1016/s2215-0366(18)30427-9.
113. Karoly HC, Mueller RL, Bidwell LC, Hutchison KE. Cannabinoids and the microbiota–gut–brain axis: Emerging effects of cannabidiol and potential applications to alcohol use disorders. *Alcohol Clin Exp Res*. 2020;44(2):340-353. https://doi.org/10.1111/acer.14256.
114. Stevens AK, Aston ER, Gunn RL, et al. Does the combination matter? Examining the influence of alcohol and cannabis product combinations on simultaneous use and consequences in daily life. *Alcohol Clin Exp Res*. 2021;45(1):181-193. https://doi.org/10.1111/acer.14494.
115. Pacula RL, Sevigny EL. Marijuana liberalizations policies: Why we can't learn much from policy still in motion. *J Policy Anal Manag*. 2014;33(1):212-221. https://doi.org/10.1002/pam.21726.

116. Terry-McElrath YM, O'Malley PM, Johnston LD. Simultaneous alcohol and marijuana use among US high school seniors from 1976 to 2011: Trends, reasons, and situations. *Drug Alcohol Depend.* 2013;133(1):71-79. https://doi.org/10.1016/j.drugalcdep.2013.05.031.
117. Waddell JT, Gunn RL, Corbin WR, Borsari B, Metrik J. Impulsive personality traits drinking less on cannabis use days: The moderating role of UPPS-P impulsive personality traits. *Psychol Addict Behav.* 2021;35(6):737-748. https://doi.org/10.1037/adb0000727.
118. Kunos G. Interactions between alcohol and the endocannabinoid system. *Alcohol Clin Exp Res.* 2020;44(4):790-805. https://doi.org/10.1111/acer.14306.
119. Bottorff JL, Bissell LJL, Balneaves LG, Oliffe JL, Capler NR, Buxton J. Perceptions of cannabis as a stigmatized medicine: A qualitative descriptive study. *Harm Reduct J.* 2013;10:2. https://doi.org/10.1186/1477-7517-10-2

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2022;42(1):03 | https://doi.org/10.35946/arcr.v42.1.03 Published: 27 January 2022

The Synaptic Interactions of Alcohol and the Endogenous Cannabinoid System

Sarah A. Wolfe, Valentina Vozella, and Marisa Roberto

Department of Molecular Medicine, Scripps Research Institute, La Jolla, California

Correspondence
Address correspondence concerning this article to Marisa Roberto, Ph.D., Department of Molecular Medicine, Scripps Research Institute, 10550 N. Torrey Pines Road, SR-305, La Jolla, CA 92037. Email: mroberto@scripps.edu

Acknowledgments
This article was supported by the National Institute on Alcohol Abuse and Alcoholism (grants AA027700, AA013498, P60 AA006420, AA017447, AA021491, AA015566, and T32 AA007456) as well as by the Pearson Center for Alcoholism and Addiction Research.

Disclosures
Dr. Roberto is *Neuropharmacology* senior section editor. The authors declare no competing financial or nonfinancial interests.

Publisher's Note
Opinions expressed in contributed articles do not necessarily reflect the views of the National Institute on Alcohol Abuse and Alcoholism, 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.

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.

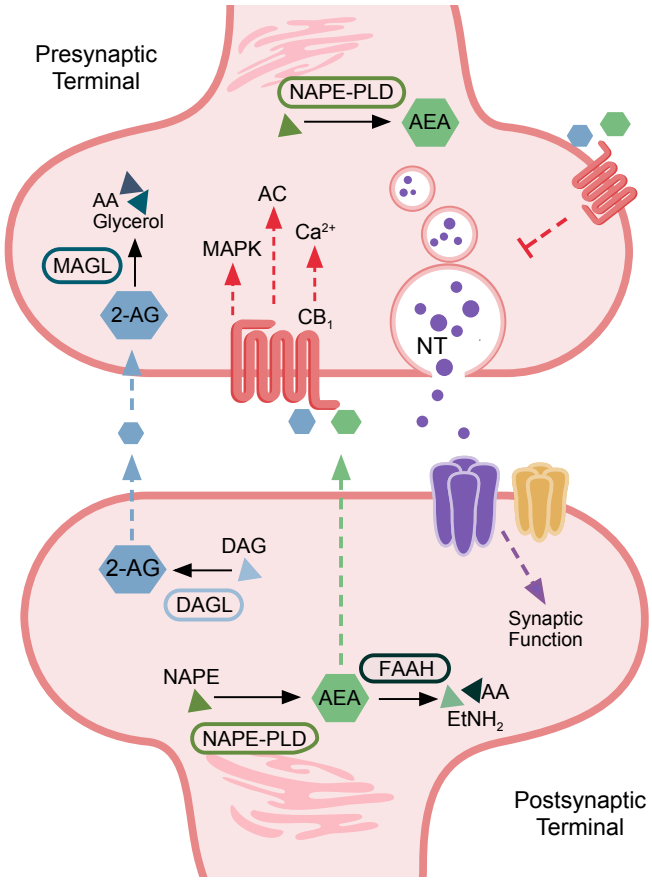

Figure 1. Summary schematic of endocannabinoid signaling in the synapse. A simplified description of the subcellular distribution of components of the endocannabinoid pathway is shown. Components include the major enzymes involved in regulating endocannabinoid levels (fatty acid amide hydrolase [FAAH], *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.

References

1. Baggio S, Sapin M, Khazaal Y, Studer J, Wolff H, Gmel G. Comorbidity of symptoms of alcohol and cannabis use disorders among a population-based sample of simultaneous users. Insight from a network perspective. *Int J Environ Res Public Health.* 2018;15(12):2893. https://doi.org/10.3390/ijerph15122893.
2. Iversen L. Cannabis and the brain. *Brain.* 2003;126(Pt 6):1252-1270. https://doi.org/10.1093/brain/awg143.
3. Metrik J, Gunn RL, Jackson KM, Sokolovsky AW, Borsari B. Daily patterns of marijuana and alcohol co-use among individuals with alcohol and cannabis use disorders. *Alcohol Clin Exp Res.* 2018;42(6):1096-1104. https://doi.org/10.1111/acer.13639.
4. Substance Abuse and Mental Health Services Administration. *2018 National Survey on Drug Use and Health: Women.* Rockville, MD: U.S. Department of Health and Human Services; 2020. https://www.samhsa.gov/data/sites/default/files/reports/rpt23250/5_Women_2020_01_14.pdf.
5. Chung T, Harris RA. Cannabis and alcohol: From basic science to public policy. *Alcohol Clin Exp Res.* 2019;43(9):1829-1833. https://doi.org/10.1111/acer.14144.
6. Kleczkowska P, Smaga I, Filip M, Bujalska-Zadrozny M. Cannabinoid ligands and alcohol addiction: A promising therapeutic tool or a humbug? *Neurotox Res.* 2016;29(1):173-196. https://doi.org/10.1007/s12640-015-9555-7.
7. Risso C, Boniface S, Subbaraman MS, Englund A. Does cannabis complement or substitute alcohol consumption? A systematic review of human and animal studies. *J Psychopharmacol.* 2020;34(9):938-954. https://doi.org/10.1177/0269881120919970.

8. Lucas P, Baron EP, Jikomes N. Medical cannabis patterns of use and substitution for opioids & other pharmaceutical drugs, alcohol, tobacco, and illicit substances; Results from a cross-sectional survey of authorized patients. *Harm Reduct J.* 2019;16(1):9. https://doi.org/10.1186/s12954-019-0278-6.
9. Subbaraman MS. Can cannabis be considered a substitute medication for alcohol? *Alcohol Alcohol.* 2014;49(3):292-298. https://doi.org/10.1093/alcalc/agt182.
10. Mechoulam R, Parker L. Cannabis and alcohol—a close friendship. *Trends Pharmacol Sci.* 2003;24(6):266-268. https://doi.org/10.1016/s0165-6147(03)00107-x.
11. Cruz MT, Bajo M, Schweitzer P, Roberto M. Shared mechanisms of alcohol and other drugs. *Alcohol Res Health.* 2008;31(2):137-147.
12. González S, Fernández-Ruiz J, Di Marzo V, et al. Behavioral and molecular changes elicited by acute administration of SR141716 to Delta^9-tetrahydrocannabinol-tolerant rats: An experimental model of cannabinoid abstinence. *Drug Alcohol Depend.* 2004;74(2):159-170. https://doi.org/10.1016/j.drugalcdep.2003.12.011.
13. Basavarajappa BS, Hungund BL. Neuromodulatory role of the endocannabinoid signaling system in alcoholism: An overview. *Prostaglandins Leukot Essent Fatty Acids.* 2002;66(2-3):287-299. https://doi.org/10.1054/plef.2001.0352.
14. Vinod KY, Hungund BL. Endocannabinoid lipids and mediated system: Implications for alcoholism and neuropsychiatric disorders. *Life Sci.* 2005;77(14):1569-1583. https://doi.org/10.1016/j.lfs.2005.05.041.
15. Freedland CS, Sharpe AL, Samson HH, Porrino LJ. Effects of SR141716A on ethanol and sucrose self-administration. *Alcohol Clin Exp Res.* 2001;25(2):277-282.
16. De Vries TJ, Schoffelmeer AN. Cannabinoid CB_1 receptors control conditioned drug seeking. *Trends Pharmacol Sci.* 2005;26(8):420-426. https://doi.org/10.1016/j.tips.2005.06.002.
17. Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL, Pistis M. Involvement of the endogenous cannabinoid system in the effects of alcohol in the mesolimbic reward circuit: Electrophysiological evidence in vivo. *Psychopharmacology (Berl).* 2005;183(3):368-377. https://doi.org/10.1007/s00213-005-0195-0.
18. Pava MJ, Woodward JJ. A review of the interactions between alcohol and the endocannabinoid system: Implications for alcohol dependence and future directions for research. *Alcohol.* 2012;46(3):185-204. https://doi.org/10.1016/j.alcohol.2012.01.002.
19. Basavarajappa BS, Joshi V, Shivakumar M, Subbanna S. Distinct functions of endogenous cannabinoid system in alcohol abuse disorders. *Br J Pharmacol.* 2019;176(17):3085-3109. https://doi.org/10.1111/bph.14780.
20. Kunos G. Interactions between alcohol and the endocannabinoid system. *Alcohol Clin Exp Res.* 2020;44(4):790-805. https://doi.org/10.1111/acer.14306.
21. Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol.* 1988;34(5):605-613.
22. Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M. The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci.* 1990;13(10):420-423. https://doi.org/10.1016/0166-2236(90)90124-s.
23. Brenneisen R. Chemistry and analysis of phytocannabinoids and other cannabis constituents. In: ElSohly MA, ed. *Marijuana and the Cannabinoids. Forensic Science and Medicine.* Totowa, NJ: Humana Press; 2007:17-49. https://doi.org/10.1007/978-1-59259-947-9_2.
24. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990;346(6284):561-564. https://doi.org/10.1038/346561a0.
25. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993;365(6441):61-65. https://doi.org/10.1038/365061a0.
26. Onaivi ES, Ishiguro H, Gu S, Liu QR. CNS effects of CB_2 cannabinoid receptors: Beyond neuro-immuno-cannabinoid activity. *J Psychopharmacol.* 2012;26(1):92-103. https://doi.org/10.1177/0269881111400652.
27. Van Sickle MD, Duncan M, Kingsley PJ, et al. Identification and functional characterization of brainstem cannabinoid CB_2 receptors. *Science.* 2005;310(5746):329-332. https://doi.org/10.1126/science.1115740.
28. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev.* 2003;83(3):1017-1066. https://doi.org/10.1152/physrev.00004.2003.
29. Lu Y, Anderson HD. Cannabinoid signaling in health and disease. *Can J Physiol Pharmacol.* 2017;95(4):311-327. https://doi.org/10.1139/cjpp-2016-0346.
30. Buckley NE, McCoy KL, Mezey E, et al. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur J Pharmacol.* 2000;396(2-3):141-149. https://doi.org/10.1016/s0014-2999(00)00211-9.
31. Zhang HY, Gao M, Shen H, et al. Expression of functional cannabinoid CB_2 receptor in VTA dopamine neurons in rats. *Addict Biol.* 2017;22(3):752-765. https://doi.org/10.1111/adb.12367.
32. Basavarajappa BS. Fetal alcohol spectrum disorder: Potential role of endocannabinoids signaling. *Brain Sci.* 2015;5(4):456-493. https://doi.org/10.3390/brainsci5040456.
33. Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992;258(5090):1946-1949. https://doi.org/10.1126/science.1470919.
34. Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci.* 2003;4(11):873-884. https://doi.org/10.1038/nrn1247.
35. Mechoulam R, Ben-Shabat S, Hanus L, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol.* 1995;50(1):83-90. https://doi.org/10.1016/0006-2952(95)00109-d.
36. Sugiura T, Kondo S, Sukagawa A, et al. 2-Arachidonoylglycerol: A possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun.* 1995;215(1):89-97. https://doi.org/10.1006/bbrc.1995.2437.
37. Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat.* 2000;61(1-2):3-18. https://doi.org/10.1016/s0090-6980(00)00051-4.
38. Irving A, Abdulrazzaq G, Chan SLF, Penman J, Harvey J, Alexander SPH. Cannabinoid receptor-related orphan G protein-coupled receptors. *Adv Pharmacol.* 2017;80:223-247. https://doi.org/10.1016/bs.apha.2017.04.004.
39. Iannotti FA, Di Marzo V, Petrosino S. Endocannabinoids and endocannabinoid-related mediators: Targets, metabolism and role in neurological disorders. *Prog Lipid Res.* 2016;62:107-128. https://doi.org/10.1016/j.plipres.2016.02.002.
40. Pistis M, Melis M. From surface to nuclear receptors: The endocannabinoid family extends its assets. *Curr Med Chem.* 2010;17(14):1450-1467. https://doi.org/10.2174/092986710790980014.
41. Basavarajappa BS, Shivakumar M, Joshi V, Subbanna S. Endocannabinoid system in neurodegenerative disorders. *J Neurochem.* 2017;142(5):624-648. https://doi.org/10.1111/jnc.14098.
42. Hussain Z, Uyama T, Tsuboi K, Ueda N. Mammalian enzymes responsible for the biosynthesis of *N*-acylethanolamines. *Biochim Biophys Acta Mol Cell Biol Lipids.* 2017;1862(12):1546-1561. https://doi.org/10.1016/j.bbalip.2017.08.006.
43. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature.* 1996;384(6604):83-87. https://doi.org/10.1038/384083a0.

44. Bisogno T, Howell F, Williams G, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol.* 2003;163(3):463-468. https://doi.org/10.1083/jcb.200305129.
45. Labar G, Wouters J, Lambert DM. A review on the monoacylglycerol lipase: At the interface between fat and endocannabinoid signalling. *Curr Med Chem.* 2010;17(24):2588-2607. https://doi.org/10.2174/092986710791859414.
46. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol.* 2007;14(12):1347-1356. https://doi.org/10.1016/j.chembiol.2007.11.006.
47. Kreitzer AC, Regehr WG. Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J Neurosci.* 2001;21(20):RC174. https://doi.org/10.1523/jneurosci.21-20-j0005.2001.
48. Wilson RI, Kunos G, Nicoll RA. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron.* 2001;31(3):453-462. https://doi.org/10.1016/s0896-6273(01)00372-5.
49. Yoshida T, Hashimoto K, Zimmer A, Maejima T, Araishi K, Kano M. The cannabinoid CB_1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar Purkinje cells. *J Neurosci.* 2002;22(5):1690-1697. https://doi.org/10.1523/jneurosci.22-05-01690.2002.
50. Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron.* 2001;29(3):717-727. https://doi.org/10.1016/s0896-6273(01)00246-x.
51. Gerdeman G, Lovinger DM. CB_1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J Neurophysiol.* 2001;85(1):468-471. https://doi.org/10.1152/jn.2001.85.1.468.
52. Szabo B, Dorner L, Pfreundtner C, Norenberg W, Starke K. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience.* 1998;85(2):395-403. https://doi.org/10.1016/s0306-4522(97)00597-6.
53. Katona I, Sperlagh B, Sik A, et al. Presynaptically located CB_1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci.* 1999;19(11):4544-4558. https://doi.org/10.1523/jneurosci.19-11-04544.1999.
54. Roberto M, Cruz M, Bajo M, Siggins GR, Parsons LH, Schweitzer P. The endocannabinoid system tonically regulates inhibitory transmission and depresses the effect of ethanol in central amygdala. *Neuropsychopharmacology.* 2010;35(9):1962-1972. https://doi.org/10.1038/npp.2010.70.
55. Varodayan FP, Soni N, Bajo M, et al. Chronic ethanol exposure decreases CB_1 receptor function at GABAergic synapses in the rat central amygdala. *Addict Biol.* 2016;21(4):788-801. https://doi.org/10.1111/adb.12256.
56. Rodríguez JJ, Mackie K, Pickel VM. Ultrastructural localization of the CB_1 cannabinoid receptor in mu-opioid receptor patches of the rat caudate putamen nucleus. *J Neurosci.* 2001;21(3):823-833. https://doi.org/10.1523/jneurosci.21-03-00823.2001.
57. Varma N, Carlson GC, Ledent C, Alger BE. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci.* 2001;21(24):RC188. https://doi.org/10.1523/jneurosci.21-24-j0003.2001.
58. Ohno-Shosaku T, Hashimotodani Y, Ano M, Takeda S, Tsubokawa H, Kano M. Endocannabinoid signalling triggered by NMDA receptor-mediated calcium entry into rat hippocampal neurons. *J Physiol.* 2007;584(Pt 2):407-418. https://doi.org/10.1113/jphysiol.2007.137505.
59. Stella N, Piomelli D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur J Pharmacol.* 2001;425(3):189-196. https://doi.org/10.1016/s0014-2999(01)01182-7.
60. Mackie K, Lai Y, Westenbroek R, Mitchell R. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci.* 1995;15(10):6552-6561. https://doi.org/10.1523/jneurosci.15-10-06552.1995.
61. Azad SC, Eder M, Marsicano G, Lutz B, Zieglgänsberger W, Rammes G. Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. *Learn Mem.* 2003;10(2):116-128. https://doi.org/10.1101/lm.53303.
62. Daniel H, Rancillac A, Crepel F. Mechanisms underlying cannabinoid inhibition of presynaptic Ca^{2+} influx at parallel fibre synapses of the rat cerebellum. *J Physiol.* 2004;557(Pt 1):159-174. https://doi.org/10.1113/jphysiol.2004.063263.
63. Schweitzer P. Cannabinoids decrease the K^+ M-current in hippocampal CA1 neurons. *J Neurosci.* 2000;20(1):51-58. https://doi.org/10.1523/jneurosci.20-01-00051.2000.
64. Moore SD, Madamba SG, Siggins GR. Ethanol diminishes a voltage-dependent K^+ current, the M-current, in CA1 hippocampal pyramidal neurons in vitro. *Brain Res.* 1990;516(2):222-228. https://doi.org/10.1016/0006-8993(90)90922-x.
65. Chevaleyre V, Heifets BD, Kaeser PS, Südhof TC, Castillo PE. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1alpha. *Neuron.* 2007;54(5):801-812. https://doi.org/10.1016/j.neuron.2007.05.020.
66. Chevaleyre V, Castillo PE. Heterosynaptic LTD of hippocampal GABAergic synapses: A novel role of endocannabinoids in regulating excitability. *Neuron.* 2003;38(3):461-472. https://doi.org/10.1016/s0896-6273(03)00235-6.
67. Castillo PE, Younts TJ, Chavez AE, Hashimotodani Y. Endocannabinoid signaling and synaptic function. *Neuron.* 2012;76(1):70-81. https://doi.org/10.1016/j.neuron.2012.09.020.
68. Stempel AV, Stumpf A, Zhang HY, et al. Cannabinoid type 2 receptors mediate a cell type-specific plasticity in the hippocampus. *Neuron.* 2016;90(4):795-809. https://doi.org/10.1016/j.neuron.2016.03.034.
69. Friedman E, Gershon S. Effect of delta^8-THC on alcohol-induced sleeping time in the rat. *Psychopharmacologia.* 1974;39(3):193-198. https://doi.org/10.1007/bf00421026.
70. Marks DF, MacAvoy MG. Divided attention performance in cannabis users and non-users following alcohol and cannabis separately and in combination. *Psychopharmacology (Berl).* 1989;99(3):397-401. https://doi.org/10.1007/bf00445566.
71. da Silva GE, Morato GS, Takahashi RN. Rapid tolerance to Delta^9-tetrahydrocannabinol and cross-tolerance between ethanol and Delta^9-tetrahydrocannabinol in mice. *Eur J Pharmacol.* 2001;431(2):201-207. https://doi.org/10.1016/s0014-2999(01)01449-2.
72. Lemos JI, Takahashi RN, Morato GS. Effects of SR141716 and WIN 55,212-2 on tolerance to ethanol in rats using the acute and rapid procedures. *Psychopharmacology (Berl).* 2007;194(2):139-149. https://doi.org/10.1007/s00213-007-0804-1.
73. Henderson-Redmond AN, Guindon J, Morgan DJ. Roles for the endocannabinoid system in ethanol-motivated behavior. *Prog Neuropsychopharmacol Biol Psychiatry.* 2016;65:330-339. https://doi.org/10.1016/j.pnpbp.2015.06.011.
74. Arnone M, Maruani J, Chaperon F, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB_1) receptors. *Psychopharmacology (Berl).* 1997;132(1):104-106. https://doi.org/10.1007/s002130050326.
75. Colombo G, Agabio R, Fà M, et al. Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. *Alcohol Alcohol.* 1998;33(2):126-130. https://doi.org/10.1093/oxfordjournals.alcalc.a008368.
76. Femenia T, García-Gutiérrez MS, Manzanares J. CB1 receptor blockade decreases ethanol intake and associated neurochemical changes in fawn-hooded rats. *Alcohol Clin Exp Res.* 2010;34(1):131-141. https://doi.org/10.1111/j.1530-0277.2009.01074.x.

77. Gallate JE, McGregor IS. The motivation for beer in rats: Effects of ritanserin, naloxone and SR 141716. *Psychopharmacology (Berl).* 1999;142(3):302-308. https://doi.org/10.1007/s002130050893.
78. Lallemand F, De Witte P. SR147778, a CB1 cannabinoid receptor antagonist, suppresses ethanol preference in chronically alcoholized Wistar rats. *Alcohol.* 2006;39(3):125-134. https://doi.org/10.1016/j.alcohol.2006.08.001.
79. Vinod KY, Yalamanchili R, Thanos PK, et al. Genetic and pharmacological manipulations of the CB_1 receptor alter ethanol preference and dependence in ethanol preferring and nonpreferring mice. *Synapse.* 2008;62(8):574-581. https://doi.org/10.1002/syn.20533.
80. Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB_1 receptor agonist. *Eur J Pharmacol.* 1999;370(3):233-240. https://doi.org/10.1016/s0014-2999(99)00170-3.
81. Kelaï S, Hanoun N, Aufrère G, Beaugè F, Hamon M, Lanfumey L. Cannabinoid-serotonin interactions in alcohol-preferring vs. alcohol-avoiding mice. *J Neurochem.* 2006;99(1):308-320. https://doi.org/10.1111/j.1471-4159.2006.04054.x.
82. Martín-Sánchez A, Warnault V, Montagud-Romero S, et al. Alcohol-induced conditioned place preference is modulated by CB2 cannabinoid receptors and modifies levels of endocannabinoids in the mesocorticolimbic system. *Pharmacol Biochem Behav.* 2019;183:22-31. https://doi.org/10.1016/j.pbb.2019.06.007.
83. Hungund BL, Basavarajappa BS, Vadasz C, et al. Ethanol, endocannabinoids, and the cannabinoidergic signaling system. *Alcohol Clin Exp Res.* 2002;26(4):565-574.
84. Hansson AC, Bermúdez-Silva FJ, Malinen H, et al. Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology.* 2007;32(1):117-126. https://doi.org/10.1038/sj.npp.1301034.
85. Zhou Y, Schwartz BI, Giza J, Gross SS, Lee FS, Kreek MJ. Blockade of alcohol escalation and "relapse" drinking by pharmacological FAAH inhibition in male and female C57BL/6J mice. *Psychopharmacology (Berl).* 2017;234(19):2955-2970. https://doi.org/10.1007/s00213-017-4691-9.
86. Cippitelli A, Bilbao A, Gorriti MA, et al. The anandamide transport inhibitor AM404 reduces ethanol self-administration. *Eur J Neurosci.* 2007;26(2):476-486. https://doi.org/10.1111/j.1460-9568.2007.05665.x.
87. Soria-Gomez E, Pagano Zottola AC, Mariani Y, et al. Subcellular specificity of cannabinoid effects in striatonigral circuits. *Neuron.* 2021;109(9):1513-1526.E11. https://doi.org/10.1016/j.neuron.2021.03.007.
88. Lovinger DM, Roberto M. Synaptic effects induced by alcohol. *Curr Top Behav Neurosci.* 2013;13:31-86. https://doi.org/10.1007/7854_2011_143.
89. Cui C, Koob GF. Titrating tipsy targets: The neurobiology of low-dose alcohol. *Trends Pharmacol Sci.* 2017;38(6):556-568. https://doi.org/10.1016/j.tips.2017.03.002.
90. Staples MC, Mandyam CD. Thinking after drinking: Impaired hippocampal-dependent cognition in human alcoholics and animal models of alcohol dependence. *Front Psychiatry.* 2016;7:162. https://doi.org/10.3389/fpsyt.2016.00162.
91. Ferrer B, Bermúdez-Silva FJ, Bilbao A, et al. Regulation of brain anandamide by acute administration of ethanol. *Biochem J.* 2007;404(1):97-104. https://doi.org/10.1042/bj20061898.
92. Rubio M, de Miguel R, Fernández-Ruiz J, Gutierrez-Lopez D, Carai MA, Ramos JA. Effects of a short-term exposure to alcohol in rats on FAAH enzyme and CB_1 receptor in different brain areas. *Drug Alcohol Depend.* 2009;99(1-3):354-358. https://doi.org/10.1016/j.drugalcdep.2008.08.004.
93. Rubio M, McHugh D, Fernández-Ruiz J, Bradshaw H, Walker JM. Short-term exposure to alcohol in rats affects brain levels of anandamide, other *N*-acylethanolamines and 2-arachidonoyl-glycerol. Neurosci Lett. 2007;421(3):270-274. https://doi.org/10.1016/j.neulet.2007.05.052.
94. Basavarajappa BS, Ninan I, Arancio O. Acute ethanol suppresses glutamatergic neurotransmission through endocannabinoids in hippocampal neurons. *J Neurochem.* 2008;107(4):1001-1013. https://doi.org/10.1111/j.1471-4159.2008.05685.x.
95. Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci.* 1996;16(14):4322-4334. https://doi.org/10.1523/jneurosci.16-14-04322.1996.
96. Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci U S A.* 1992;89(9):3825-3829. https://doi.org/10.1073/pnas.89.9.3825.
97. Twitchell W, Brown S, Mackie K. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol.* 1997;78(1):43-50. https://doi.org/10.1152/jn.1997.78.1.43.
98. Johnston JB. Further contributions to the study of the evolution of the forebrain. V. Survey of forebrain morphology. *J Comp Neurol.* 1923;36(2):143-192. https://doi.org/10.1002/cne.900360205.
99. Heimer L, Alheid GF. Piecing together the puzzle of basal forebrain anatomy. *Adv Exp Med Biol.* 1991;295:1-42. https://doi.org/10.1007/978-1-4757-0145-6_1.
100. Koob GF, Le Moal M. Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. *Nat Neurosci.* 2005;8(11):1442-1444. https://doi.org/10.1038/nn1105-1442.
101. Roberto M, Kirson D, Khom S. The role of the central amygdala in alcohol dependence. *Cold Spring Harb Perspect Med.* 2021;11(2):a039339. https://doi.org/10.1101/cshperspect.a039339.
102. Koob GF. Drug addiction: Hyperkatifeia/negative reinforcement as a framework for medications development. *Pharmacol Rev.* 2021;73(1):163-201. https://doi.org/10.1124/pharmrev.120.000083.
103. Roberto M, Schweitzer P, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: An in vitro and in vivo analysis. *J Neurosci.* 2004;24(7):1594-1603. https://doi.org/10.1523/jneurosci.5077-03.2004.
104. Roberto M, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Increased GABA release in the central amygdala of ethanol-dependent rats. *J Neurosci.* 2004;24(45):10159-10166. https://doi.org/10.1523/jneurosci.3004-04.2004.
105. Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc Natl Acad Sci U S A.* 2003;100(4):2053-2058. https://doi.org/10.1073/pnas.0437926100.
106. Ramikie TS, Nyilas R, Bluett RJ, et al. Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. *Neuron.* 2014;81(5):1111-1125. https://doi.org/10.1016/j.neuron.2014.01.012.
107. Kirson D, Oleata CS, Parsons LH, Ciccocioppo R, Roberto M. CB_1 and ethanol effects on glutamatergic transmission in the central amygdala of male and female msP and Wistar rats. *Addict Biol.* 2018;23(2):676-688. https://doi.org/10.1111/adb.12525.
108. Serrano A, Parsons LH. Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol Ther.* 2011;132(3):215-241. https://doi.org/10.1016/j.pharmthera.2011.06.005.
109. Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology.* 2016;41(1):80-102. https://doi.org/10.1038/npp.2015.166.
110. Katona I, Rancz EA, Acsády L, et al. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci.* 2001;21(23):9506-9518. https://doi.org/10.1523/jneurosci.21-23-09506.2001.

111. Yoshida T, Uchigashima M, Yamasaki M, et al. Unique inhibitory synapse with particularly rich endocannabinoid signaling machinery on pyramidal neurons in basal amygdaloid nucleus. *Proc Natl Acad Sci U S A*. 2011;108(7):3059-3064. https://doi.org/10.1073/pnas.1012875108.
112. Marsicano G, Wotjak CT, Azad SC, et al. The endogenous cannabinoid system controls extinction of aversive memories. *Nature*. 2002;418(6897):530-534. https://doi.org/10.1038/nature00839.
113. Azad SC, Monory K, Marsicano G, et al. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. *J Neurosci*. 2004;24(44):9953-9961. https://doi.org/10.1523/jneurosci.2134-04.2004.
114. Zhu PJ, Lovinger DM. Retrograde endocannabinoid signaling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. *J Neurosci*. 2005;25(26):6199-6207. https://doi.org/10.1523/jneurosci.1148-05.2005.
115. Varodayan FP, Bajo M, Soni N, et al. Chronic alcohol exposure disrupts CB_1 regulation of GABAergic transmission in the rat basolateral amygdala. *Addict Biol*. 2017;22(3):766-778. https://doi.org/10.1111/adb.12369.
116. Perra S, Pillolla G, Luchicchi A, Pistis M. Alcohol inhibits spontaneous activity of basolateral amygdala projection neurons in the rat: Involvement of the endocannabinoid system. *Alcohol Clin Exp Res*. 2008;32(3):443-449. https://doi.org/10.1111/j.1530-0277.2007.00588.x.
117. Talani G, Lovinger DM. Interactions between ethanol and the endocannabinoid system at GABAergic synapses on basolateral amygdala principal neurons. *Alcohol*. 2015;49(8):781-794. https://doi.org/10.1016/j.alcohol.2015.08.006.
118. Roberto M, Varodayan FP. Synaptic targets: Chronic alcohol actions. *Neuropharmacology*. 2017;122:85-99. https://doi.org/10.1016/j.neuropharm.2017.01.013.
119. Xu L, Nan J, Lan Y. The nucleus accumbens: A common target in the comorbidity of depression and addiction. *Front Neural Circuits*. 2020;14:37. https://doi.org/10.3389/fncir.2020.00037.
120. Ceccarini J, Casteels C, Koole M, Bormans G, Van Laere K. Transient changes in the endocannabinoid system after acute and chronic ethanol exposure and abstinence in the rat: A combined PET and microdialysis study. *Eur J Nucl Med Mol Imaging*. 2013;40(10):1582-1594. https://doi.org/10.1007/s00259-013-2456-1.
121. Subbanna S, Shivakumar M, Psychoyos D, Xie S, Basavarajappa BS. Anandamide-CB_1 receptor signaling contributes to postnatal ethanol-induced neonatal neurodegeneration, adult synaptic, and memory deficits. *J Neurosci*. 2013;33(15):6350-6366. https://doi.org/10.1523/jneurosci.3786-12.2013.
122. Caillé S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH. Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. *J Neurosci*. 2007;27(14):3695-3702.
123. Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C. Cannabinoid CB_1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J Neurochem. 2003;84(4):698-704*. https://doi.org/10.1046/j.1471-4159.2003.01576.x.
124. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A*. 1988;85(14):5274-5278. https://doi.org/10.1073/pnas.85.14.5274.
125. Bustamante D, Quintanilla ME, Tampier L, Gonzalez-Lira V, Israel Y, Herrera-Marschitz M. Ethanol induces stronger dopamine release in nucleus accumbens (shell) of alcohol-preferring (bibulous) than in alcohol-avoiding (abstainer) rats. *Eur J Pharmacol*. 2008;591(1-3):153-158. https://doi.org/10.1016/j.ejphar.2008.06.069.
126. Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science*. 1997;276(5321):2048-2050. https://doi.org/10.1126/science.276.5321.2048.
127. Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB_1 receptors. *Eur J Pharmacol*. 1998;341(1):39-44. https://doi.org/10.1016/s0014-2999(97)01442-8.
128. Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J Neurosci*. 2004;24(18):4393-4400. https://doi.org/10.1523/jneurosci.0529-04.2004.
129. Lupica CR, Riegel AC, Hoffman AF. Marijuana and cannabinoid regulation of brain reward circuits. *Br J Pharmacol*. 2004;143(2):227-234. https://doi.org/10.1038/sj.bjp.0705931.
130. Mateo Y, Johnson KA, Covey DP, et al. Endocannabinoid actions on cortical terminals orchestrate local modulation of dopamine release in the nucleus accumbens. *Neuron*. 2017;96(5):1112-1126.e5. https://doi.org/10.1016/j.neuron.2017.11.012.
131. You C, Vandegrift B, Brodie MS. Ethanol actions on the ventral tegmental area: Novel potential targets on reward pathway neurons. *Psychopharmacology (Berl)*. 2018;235(6):1711-1726. https://doi.org/10.1007/s00213-018-4875-y.
132. Clarke RB, Adermark L. Acute ethanol treatment prevents endocannabinoid-mediated long-lasting disinhibition of striatal output. *Neuropharmacology*. 2010;58(4-5):799-805. https://doi.org/10.1016/j.neuropharm.2009.12.006.
133. Valenzuela CF, Jotty K. Mini-review: Effects of ethanol on $GABA_A$ receptor-mediated neurotransmission in the cerebellar cortex—recent advances. *Cerebellum*. 2015;14(4):438-446. https://doi.org/10.1007/s12311-014-0639-3.
134. Kelm MK, Criswell HE, Breese GR. Calcium release from presynaptic internal stores is required for ethanol to increase spontaneous gamma-aminobutyric acid release onto cerebellum Purkinje neurons. *J Pharmacol Exp Ther*. 2007;323(1):356-364. https://doi.org/10.1124/jpet.107.126144.
135. Kelm MK, Criswell HE, Breese GR. The role of protein kinase A in the ethanol-induced increase in spontaneous GABA release onto cerebellar Purkinje neurons. *J Neurophysiol*. 2008;100(6):3417-3428. https://doi.org/10.1152/jn.90970.2008.
136. Vinod KY, Yalamanchili R, Xie S, Cooper TB, Hungund BL. Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid system. *Neurochem Int*. 2006;49(6):619-625. https://doi.org/10.1016/j.neuint.2006.05.002.
137. Ceccarini J, Hompes T, Verhaeghen A, et al. Changes in cerebral CB_1 receptor availability after acute and chronic alcohol abuse and monitored abstinence. *J Neurosci*. 2014;34(8):2822-2831. https://doi.org/10.1523/jneurosci.0849-13.2014.
138. Basavarajappa BS, Hungund BL. Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor *N*-arachidonoylphosphatidylethanolamine in SK-N-SH cells. *J Neurochem*. 1999;72(2):522-528. https://doi.org/10.1046/j.1471-4159.1999.0720522.x.
139. Mitrirattanakul S, López-Valdés HE, Liang J, et al. Bidirectional alterations of hippocampal cannabinoid 1 receptors and their endogenous ligands in a rat model of alcohol withdrawal and dependence. *Alcohol Clin Exp Res*. 2007;31(5):855-867. https://doi.org/10.1111/j.1530-0277.2007.00366.x.
140. Cippitelli A, Bilbao A, Hansson AC, et al. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur J Neurosci*. 2005;21(8):2243-2251. https://doi.org/10.1111/j.1460-9568.2005.04056.x.
141. Ortiz S, Oliva JM, Pérez-Rial S, Palomo T, Manzanares J. Chronic ethanol consumption regulates cannabinoid CB_1 receptor gene expression in selected regions of rat brain. *Alcohol Alcohol*. 2004;39(2):88-92. https://doi.org/10.1093/alcalc/agh036.
142. Natividad LA, Buczynski MW, Herman MA, et al. Constitutive increases in amygdalar corticotropin-releasing factor and fatty acid amide hydrolase drive an anxious phenotype. *Biol Psychiatry*. 2017;82(7):500-510. https://doi.org/10.1016/j.biopsych.2017.01.005.

143. Hirvonen J, Zanotti-Fregonara P, Umhau JC, et al. Reduced cannabinoid CB_1 receptor binding in alcohol dependence measured with positron emission tomography. *Mol Psychiatry.* 2013;18(8):916-921. https://doi.org/10.1038/mp.2012.100.
144. Pava MJ, Woodward JJ. Chronic ethanol alters network activity and endocannabinoid signaling in the prefrontal cortex. *Front Integr Neurosci.* 2014;8:58. https://doi.org/10.3389/fnint.2014.00058.
145. Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J.* 2002;16(1):27-35. https://doi.org/10.1096/fj.01-0593com.
146. Henricks AM, Berger AL, Lugo JM, et al. Sex- and hormone-dependent alterations in alcohol withdrawal-induced anxiety and corticolimbic endocannabinoid signaling. *Neuropharmacology.* 2017;124:121-133. https://doi.org/10.1016/j.neuropharm.2017.05.023.
147. Vinod KY, Maccioni P, Garcia-Gutierrez MS, et al. Innate difference in the endocannabinoid signaling and its modulation by alcohol consumption in alcohol-preferring sP rats. *Addict Biol.* 2012;17(1):62-75. https://doi.org/10.1111/j.1369-1600.2010.00299.x.
148. Roberto M, Nelson TE, Ur CL, Gruol DL. Long-term potentiation in the rat hippocampus is reversibly depressed by chronic intermittent ethanol exposure. *J Neurophysiol.* 2002;87(5):2385-2397. https://doi.org/10.1152/jn.2002.87.5.2385.
149. Roberto M, Nelson TE, Ur CL, Brunelli M, Sanna PP, Gruol DL. The transient depression of hippocampal CA1 LTP induced by chronic intermittent ethanol exposure is associated with an inhibition of the MAP kinase pathway. *Eur J Neurosci.* 2003;17(8):1646-1654. https://doi.org/10.1046/j.1460-9568.2003.02614.x.
150. Moranta D, Esteban S, García-Sevilla JA. Ethanol desensitizes cannabinoid CB_1 receptors modulating monoamine synthesis in the rat brain in vivo. *Neurosci Lett.* 2006;392(1-2):58-61. https://doi.org/10.1016/j.neulet.2005.08.061.
151. Warnault V, Houchi H, Barbier E, et al. The lack of CB1 receptors prevents neuroadapatations of both NMDA and $GABA_A$ receptors after chronic ethanol exposure. *J Neurochem.* 2007;102(3):741-752. https://doi.org/10.1111/j.1471-4159.2007.04577.x.
152. Fu R, Tang Y, Li W, et al. Endocannabinoid signaling in the lateral habenula regulates pain and alcohol consumption. *Transl Psychiatry.* 2021;11(1):220. https://doi.org/10.1038/s41398-021-01337-3.
153. González S, Fernández-Ruiz J, Sparpaglione V, Parolaro D, Ramos JA. Chronic exposure to morphine, cocaine or ethanol in rats produced different effects in brain cannabinoid CB_1 receptor binding and mRNA levels. *Drug Alcohol Depend.* 2002;66(1):77-84. https://doi.org/10.1016/s0376-8716(01)00186-7.
154. Abernathy K, Chandler LJ, Woodward JJ. Alcohol and the prefrontal cortex. *Int Rev Neurobiol.* 2010;91:289-320. https://doi.org/10.1016/S0074-7742(10)91009-X.
155. González S, Cascio MG, Fernández-Ruiz J, Fezza F, Di Marzo V, Ramos JA. Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. *Brain Res.* 2002;954(1):73-81. https://doi.org/10.1016/s0006-8993(02)03344-9.
156. Serrano A, Rivera P, Pavon FJ, et al. Differential effects of single versus repeated alcohol withdrawal on the expression of endocannabinoid system-related genes in the rat amygdala. *Alcohol Clin Exp Res.* 2012;36(6):984-994. https://doi.org/10.1111/j.1530-0277.2011.01686.x.
157. Serrano A, Pavon FJ, Buczynski MW, et al. Deficient endocannabinoid signaling in the central amygdala contributes to alcohol dependence-related anxiety-like behavior and excessive alcohol intake. *Neuropsychopharmacology.* 2018;43(9):1840-1850. https://doi.org/10.1038/s41386-018-0055-3.
158. Chevaleyre V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci.* 2006;29:37-76. https://doi.org/10.1146/annurev.neuro.29.051605.112834.
159. Silberman Y, Shi L, Brunso-Bechtold JK, Weiner JL. Distinct mechanisms of ethanol potentiation of local and paracapsular GABAergic synapses in the rat basolateral amygdala. *J Pharmacol Exp Ther.* 2008;324(1):251-260. https://doi.org/10.1124/jpet.107.128728.
160. McCool BA, Frye GD, Pulido MD, Botting SK. Effects of chronic ethanol consumption on rat $GABA_A$ and strychnine-sensitive glycine receptors expressed by lateral/basolateral amygdala neurons. *Brain Res.* 2003;963(1-2):165-177. https://doi.org/10.1016/s0006-8993(02)03966-5.
161. Diaz MR, Christian DT, Anderson NJ, McCool BA. Chronic ethanol and withdrawal differentially modulate lateral/basolateral amygdala paracapsular and local GABAergic synapses. *J Pharmacol Exp Ther.* 2011;337(1):162-170. https://doi.org/10.1124/jpet.110.177121.
162. Hill MN, Patel S, Campolongo P, Tasker JG, Wotjak CT, Bains JS. Functional interactions between stress and the endocannabinoid system: From synaptic signaling to behavioral output. *J Neurosci.* 2010;30(45):14980-14986. https://doi.org/10.1523/jneurosci.4283-10.2010.
163. Tan H, Ahmad T, Loureiro M, Zunder J, Laviolette SR. The role of cannabinoid transmission in emotional memory formation: Implications for addiction and schizophrenia. *Front Psychiatry.* 2014;5:73. https://doi.org/10.3389/fpsyt.2014.00073.
164. Robinson SL, Alexander NJ, Bluett RJ, Patel S, McCool BA. Acute and chronic ethanol exposure differentially regulate CB1 receptor function at glutamatergic synapses in the rat basolateral amygdala. *Neuropharmacology.* 2016;108:474-484. https://doi.org/10.1016/j.neuropharm.2015.12.005.
165. Harlan BA, Becker HC, Woodward JJ, Riegel AC. Opposing actions of CRF-R1 and CB1 receptors on VTA-GABAergic plasticity following chronic exposure to ethanol. *Neuropsychopharmacology.* 2018;43(10):2064-2074. https://doi.org/10.1038/s41386-018-0106-9.
166. Vinod KY, Kassir SA, Hungund BL, Cooper TB, Mann JJ, Arango V. Selective alterations of the CB_1 receptors and the fatty acid amide hydrolase in the ventral striatum of alcoholics and suicides. *J Psychiatr Res.* 2010;44(9):591-597. https://doi.org/10.1016/j.jpsychires.2009.11.013.
167. DePoy L, Daut R, Brigman JL, et al. Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. *Proc Natl Acad Sci U S A.* 2013;110(36):14783-14788. https://doi.org/10.1073/pnas.1308198110.
168. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. *Biochim Biophys Acta.* 2000;1535(1):78-86. https://doi.org/10.1016/s0925-4439(00)00085-5.
169. Basavarajappa BS, Cooper TB, Hungund BL. Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. *Brain Res.* 1998;793(1-2):212-218. https://doi.org/10.1016/s0006-8993(98)00175-9.
170. Basavarajappa BS, Hungund BL. Down-regulation of cannabinoid receptor agonist-stimulated [^{35}S]GTP gamma S binding in synaptic plasma membrane from chronic ethanol exposed mouse. *Brain Res.* 1999;815(1):89-97. https://doi.org/10.1016/s0006-8993(98)01072-5.
171. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. *Eur J Pharmacol.* 2003;466(1-2):73-83. https://doi.org/10.1016/s0014-2999(03)01557-7.
172. Karoly HC, Mueller RL, Andrade CC, Hutchison KE. THC and CBD effects on alcohol use among alcohol and cannabis co-users. *Psychol Addict Behav.* 2021. https://doi.org/10.1037/adb0000706.

173. Gunn R, Jackson K, Borsari B, Metrik J. A longitudinal examination of daily patterns of cannabis and alcohol co-use among medicinal and recreational veteran cannabis users. *Drug Alcohol Depend.* 2019;205:107661. https://doi.org/10.1016/j.drugalcdep.2019.107661.
174. Stopponi S, Fotio Y, Domi A, et al. Inhibition of fatty acid amide hydrolase in the central amygdala alleviates co-morbid expression of innate anxiety and excessive alcohol intake. *Addict Biol.* 2018;23(6):1223-1232. https://doi.org/10.1111/adb.12573.
175. Cippitelli A, Cannella N, Braconi S, et al. Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology (Berl).* 2008;198(4):449-460. https://doi.org/10.1007/s00213-008-1104-0.
176. Holleran KM, Wilson HH, Fetterly TL, et al. Ketamine and MAG lipase inhibitor-dependent reversal of evolving depressive-like behavior during forced abstinence from alcohol drinking. *Neuropsychopharmacology.* 2016;41(8):2062-2071. https://doi.org/10.1038/npp.2016.3.
177. Fucich EA, Mayeux JP, McGinn MA, Gilpin NW, Edwards S, Molina PE. A novel role for the endocannabinoid system in ameliorating motivation for alcohol drinking and negative behavioral affect after traumatic brain injury in rats. *J Neurotrauma.* 2019;36(11):1847-1855. https://doi.org/10.1089/neu.2018.5854.

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2021;41(1):12 | https://doi.org/10.35946/arcr.v41.1.12 Published: 23 September 2021

Hepatic Cannabinoid Signaling in the Regulation of Alcohol-Associated Liver Disease

Keungmo Yang,[1] Sung Eun Choi,[1] and Won-Il Jeong[1,2]

[1]Laboratory of Liver Research, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

[2]Biomedical Research Center, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

Correspondence
Address correspondence concerning this article to Won-Il Jeong, D.V.M., Ph.D., Laboratory of Liver Research, Building E7, Room 8107, GSMSE/KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea. Email: wijeong@kaist.ac.kr

Acknowledgments
This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government Ministry of Science and Information and Communications Technology (2018R1A2A1A05077608 and 2021R1A3B1076878); Global Ph.D. Fellowship Program (NRF-2019H1A2A1074222); Korea Mouse Phenotyping Project (2014M3A9D5A01073556); and KAIST Grand Challenge 30 Project (N11210112).

Disclosures
The authors declare no competing financial or nonfinancial interests.

PURPOSE: The endocannabinoid system has emerged as a key regulatory signaling pathway in the pathophysiology of alcohol-associated liver disease (ALD). More than 30 years of research have established different roles of endocannabinoids and their receptors in various aspects of liver diseases, such as steatosis, inflammation, and fibrosis. However, pharmacological applications of the endocannabinoid system for the treatment of ALD have not been successful because of psychoactive side effects, despite some beneficial effects. Thus, a more delicate and detailed elucidation of the mechanism linking the endocannabinoid system and ALD may be of paramount significance in efforts to apply the system to the treatment of ALD.
SEARCH METHODS: Three electronic databases (PubMed, MEDLINE, and Cochrane Library) were used for literature search from November 1988 to April 2021. Major keywords used for literature searches were "cannabinoid," "cannabinoid receptor," "ALD," "steatosis," and "fibrosis."
SEARCH RESULTS: According to the inclusion and exclusion criteria, the authors selected 47 eligible full-text articles out of 2,691 searched initially. Studies in the past 3 decades revealed the opposite effects of cannabinoid receptors CB1R and CB2R on steatosis, inflammation, and fibrosis in ALD.
DISCUSSION AND CONCLUSIONS: This review summarizes the endocannabinoid signaling in the general physiology of the liver, the pathogenesis of ALD, and some of the potential therapeutic implications of cannabinoid-based treatments for ALD.

KEYWORDS: alcohol; CB1R; CB2R; cell communication; endocannabinoid; fatty liver; metabotropic glutamate receptor 5; xCT

The prevalence of alcohol use disorder has been steadily rising around the world in recent years, and reducing the burden of alcohol-associated liver disease (ALD) caused by chronic alcohol consumption has become one of the most important global health issues.[1,2] Excessive alcohol drinking (more than 40 g of pure alcohol per day) is closely associated with increased risk of all-cause mortality including chronic diseases, such as cancer, cardiovascular conditions, and neuronal diseases.[3] ALD comprises a wide spectrum of liver injury including simple steatosis, steatohepatitis, liver cirrhosis, and hepatocellular carcinoma. The predominant cause of alcohol-associated liver disease, as evident by its name, is the persistent intake of alcohol, and yet the detailed mechanisms of ALD progression remain vague.[4,5]

ALD develops through complex signaling pathways in the liver.[6] Chronic alcohol consumption not only elicits various responses by innate immune cells in the liver, but also contributes to the metabolic dysfunction of hepatocytes, such as the production of reactive oxygen species (ROS), the abnormal lipogenesis induced by endoplasmic reticulum stress or mitochondrial dysfunction, and the secretion of inflammatory cytokines.[6] Apart from alcohol-induced effects, endogenous cannabinoids (endocannabinoids), which are lipid mediators, also were found to play an important role in provoking ethanol-induced hepatic steatosis.[7] The study of endocannabinoids began with the discovery that delta 9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis, binds to G-protein-coupled receptors and exhibits diverse biological effects in the brain depending on the types of functioning cells affected.[8] Over the past 3 decades, mounting evidence has shown that in peripheral organs, endocannabinoids modulate the progression of various diseases including nonalcoholic fatty liver disease (NAFLD), liver fibrosis, and ALD.[9] However, the underlying mechanisms and the specifics of the cannabinoid signaling are yet to be elucidated. The authors of this review recently reported, however, that alcoholic steatosis is promoted by endocannabinoid production in hepatic stellate cells (HSCs), which is mediated by metabotropic glutamate receptor 5 (mGluR5).[10] This review explores cannabinoid signaling in regard to the general physiology of hepatic function, the pathogenesis of ALD, and the potential therapeutic implications for ALD.

Methods and Results of the Literature Search

In-depth literature investigation was performed for this review article. Three online databases (PubMed, MEDLINE, and Cochrane Library) were used for literature search. The major search terms used were "cannabinoid," "endocannabinoid," "cannabinoid receptor," "alcoholic liver disease," "steatosis," and "fibrosis." Among the initial search results retrieved from the online databases, articles published later than April 2021 and duplicate articles were removed, and articles written in English were screened first. Then, the authors included peer-reviewed original articles on animal experiments or clinical trials and well-organized review articles relevant to the subject. Research articles without peer review, abstracts of conferences or posters, and articles with unclear research processes or insufficient data were excluded. As a result, 47 eligible full-text articles were selected from a total of 2,691 searched initially. All authors independently conducted literature searches using the same online databases, and then selected appropriate references according to the inclusion and exclusion criteria.

Cannabinoid Signaling Systems and Hepatic Function

Endocannabinoid System

Marijuana (*Cannabis sativa*) has been widely used for medical applications (e.g., analgesic, antiemetic, appetite stimulant) since its discovery in ancient times.[11] Now it is better known to the public for its psychoactive effects such as euphoria, relaxation, increased awareness of sensation, and alteration of conscious perception.[12] Among the 60 different ingredients of marijuana, early research focused on THC, a phytocannabinoid, as it has the strongest psychoactive property. Because of its highly lipophilic and hydrophobic properties, THC was believed to provoke its effects nonspecifically by perturbing the membrane phospholipids. This misunderstanding persisted until the revelation of two cannabinoid receptors: type 1 (CB1R) and type 2 (CB2R).[13]

In comparison to their expression in the central nervous system (CNS), such as in the brain and spine, CB1R and CB2R are relatively less distributed and work differently in peripheral organs.[14,15] For instance, CB1R and its ligands have critical roles in the pathogenesis of chronic liver diseases, such as steatosis and liver fibrosis.[14,15] Meanwhile, CB2R is mainly distributed in immune cells or hematopoietic organs, where it functions as a protective responder to specific pathological conditions, especially in liver fibrosis.[16,17] Like marijuana, endocannabinoids generally consist of analogs of long-chain polyunsaturated fatty acids and have an arachidonic acid moiety that confers a strong affinity with cannabinoid receptors.[18] The two most extensively studied endocannabinoids are arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG).[18]

The components and signaling pathways of the endocannabinoid system are similar in most organs throughout the body.[18] As endogenous or exogenous cannabinoids arrive at target cells, both CB1R and CB2R are stimulated with heterotrimeric G-proteins and suppress adenylate cyclase to inhibit the phosphorylation of protein kinase A. In contrast, mitogen-activated protein kinase is stimulated to regulate additional gene expressions.[14,18] In the case of CB1R, when heterodimeric G-protein is stimulated, it directly inhibits the membrane's calcium channels and stimulates the potassium channels to inhibit the release of neurotransmitters in neuronal cells.[14] However, the activation of cannabinoid receptor–mediated signaling pathways may differ depending on the type of cells stimulated.[18]

Endocannabinoid Production and Degradation

Endocannabinoids are biosynthesized through various pathways from several precursors of phospholipids located in the cellular membrane. Figure 1 schematically summarizes the biosynthesis and degradation pathways of endocannabinoids AEA and 2-AG.[11,14,19] N-arachidonoyl-phosphatidylethanolamine (NAPE), a phospholipid precursor located in the cell membrane, is preferentially synthesized from glycerophospholipid and phosphatidylethanolamine by N-acyltransferase (NAT) and sequentially hydrolyzed by the NAPE-specific phospholipase D (NAPE-PLD) in response to stimulation, subsequently resulting in the production of AEA (see Figure 1).[19] Degradation of AEA involves its hydrolysis into arachidonic acid and ethanolamine by a number of enzymes, namely fatty acid amide hydrolase (FAAH) and N-acylethanolamine-hydrolyzing acid amidase (NAAA), in the intracellular space.[20,21] As for 2-AG, *sn*-1-acyl-2-arachidonoyl-glycerol (DAG) is first produced from the intracellular glycerophospholipid by phospholipase C at the plasma membrane. Then, DAG is subsequently hydrolyzed by diacylglycerol lipase (DAGL) to 2-AG.[22] Although the chemical structures of DAGL-alpha and DAGL-beta are slightly different, their preference for ligands is similar.[14] Interestingly, a study has shown that DAGL-alpha has a more dominant role over DAGL-beta in regulating the levels of 2-AG in the brain, but the opposite was observed in the liver. In fact, only DAGL-beta, but not DAGL-alpha, has been reported to be expressed in HSCs of fatty mouse liver.[7,10] Unlike AEA, 2-AG is believed to be degraded into arachidonic acid and glycerol by several enzymes, FAAH, and monoacylglycerol lipase (MAGL).[22]

Generally, the activation of both NAPE-PLD and DAGL is triggered by changes in the intracellular calcium signaling.[12,20] When calcium influx occurs in a cell by a specific stimulus, the intracellular concentration of AEA or 2-AG increases due to the activation of endocannabinoid-producing enzymes. The newly synthesized endocannabinoids are then transported from the cytoplasm out of the cell by a specific transporter, the endocannabinoid membrane transporter.[11,21] Because of their hydrophobic properties, the released endocannabinoids have high binding affinities to the membrane, enabling them to rapidly bind to their specific receptors and induce biological responses in the neighboring cells. For instance, the AEA and 2-AG generated by the activation of endocannabinoid-producing enzymes stimulate hepatic CB1R to induce de novo lipogenesis in nonalcoholic and alcoholic fatty liver.[7,23] In general, 2-AG acts as a full agonist at these cannabinoid receptors, whereas AEA has a weaker potency as an agonist.[13] Although levels of 2-AG and AEA in peripheral tissues vary, 2-AG (~ 0.8 pmol/mg tissue) is maintained at higher levels than AEA (~ 1.1 fmol/mg tissue) in the liver.[7] In terms of alcohol-mediated endocannabinoid production, studies have demonstrated that chronic ethanol exposure or consumption induces 2-AG production in cerebellar granule neurons in vitro or in HSCs in vivo, respectively.[7,10,24]

Cannabinoid Receptor Expression

In line with their differences in synthesis, AEA and 2-AG have different affinities for their respective cannabinoid receptors.[12] AEA has a stronger affinity for CB1R than for CB2R, whereas 2-AG has a similar affinity for both CB1R and CB2R. In addition, AEA and 2-AG are also known to bind receptors other than the cannabinoid receptors, such as the transient receptor potential vanilloid type 1 (TRPV-1) and the orphan G protein-coupled receptors 55 (GPR55) and 119 (GPR119).[14,19] However, with little being known, the detailed physiological effect of endocannabinoid binding to these non-cannabinoid receptors on the cellular pathophysiology in the liver remains enigmatic.

Once the endocannabinoids, either synthetic or endogenous, bind to their cannabinoid receptors, both the CB1R and CB2R get stimulated enough to rapidly transduce extracellular signals into cells.[25, 26] With regards to their expression, they are widely distributed throughout our body as summarized in Figure 2. CB1R is predominantly distributed in the central and peripheral nervous system, including the sensorial peripheral and sympathetic nerves in humans and mice.[26] However, abundant evidence has confirmed that CB1R is also characteristically expressed in several peripheral tissues and organs, including liver, lung, gastrointestinal tract, urinary tract, thyroid, pancreas, heart, vascular endothelium, adipose tissue, reproductive organs, skeletal muscles, and immune system (see Figure 2).[11,25] Unlike CB1R, CB2R is mainly expressed in cells and organs that are responsible for controlling peripheral hematopoiesis or immune functions (see Figure 2).[25,26] For example, macrophages, neutrophils, monocytes, B lymphocytes, T lymphocytes, and microglial cells are representative of CB2R-expressing cells.

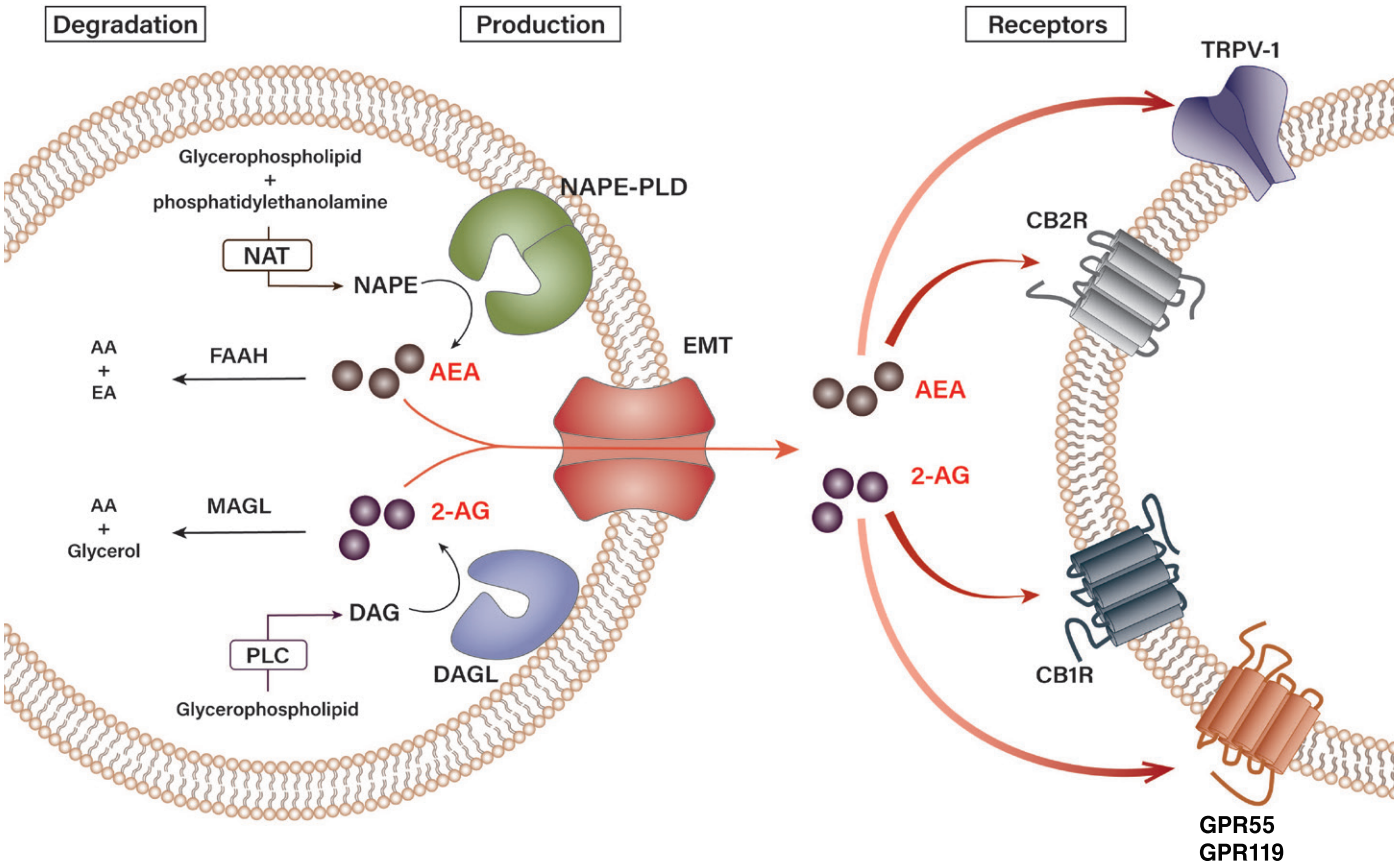

Figure 1. Biosynthesis and degradation pathways of endocannabinoids. Endogenous cannabinoids (endocannabinoids)—arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG)—have distinct pathways of synthesis and degradation in cells. N-arachidonoyl-phosphatidylethanolamine (NAPE) is synthesized from glycerophospholipid and phosphatidylethanolamine by N-acyltransferase (NAT). Upon stimulation, NAPE subsequently gets hydrolyzed by NAPE-specific phospholipase D (NAPE-PLD) to produce AEA. Synthesis of 2-AG begins with the production of *sn*-1-acyl-2-arachidonoyl-glycerol (DAG) from glycerophospholipid by phospholipase C (PLC), which is then hydrolyzed by diacylglycerol lipase (DAGL) to 2-AG. The synthesized AEA and 2-AG are transported out of the cell by an endocannabinoid membrane transporter (EMT). The released AEA and 2-AG then bind their cannabinoid and noncannabinoid receptors in the neighboring cells to transduce extracellular signals. 2-AG binds both cannabinoid-1 receptor (CB1R) and cannabinoid-2 receptor (CB2R) with similar affinity, whereas AEA has a stronger affinity for CB1R. 2-AG and AEA also bind transient receptor potential vanilloid type-1 (TRPV-1) and orphan G protein-coupled receptors 55 (GPR55) and 119 (GPR119). AEA is hydrolyzed into arachidonic acid (AA) and ethanolamine (EA) by fatty acid amide hydrolase type-1 (FAAH-1) and type-2 (FAAH-2), and N-acylethanolamine-hydrolyzing acid amidase (NAAA), whereas 2-AG is degraded into AA and glycerol by monoacylglycerol lipase (MAGL) and FAAH.

Recently, an increasing number of reports have expanded the scope of peripheral tissue known to contain CB2R to include skin nerve fibers, keratinocytes, bone cells (i.e., osteoblasts, osteocytes, and osteoclasts), and somatostatin-secreting cells in the pancreas.[27]

Cannabinoid Receptor Activation in the Liver

Early research on endocannabinoids focused on demonstrating the mechanism of psychoactive symptoms and their neurologic signals caused by the stimulation of CB1R in the brain.[13,26] However, little attention was paid to the biological roles of the hepatic endocannabinoid system despite the discovery of cannabinoid receptors in the liver.[9] Nowadays, emerging lines of evidence have shown that diverse types of the hepatic cells not only express CB1R or CB2R but also employ them in the hepatic pathophysiology, drawing attention to the critical correlation between chronic liver diseases and cannabinoid receptor signaling.[28]

Hepatocytes, the parenchymal cells of the liver, mainly express CB1R, but the level of expression is relatively low in the homeostatic condition (see Figure 2). However, CB1R expression is tremendously elevated in pathological conditions, such as alcoholic and nonalcoholic steatosis, primary biliary cirrhosis, and hepatocellular carcinoma.[9,19,29] CB2R is rarely

expressed in the steady state of the liver, but its expression is elevated in immune cells during the occurrence of hepatic regeneration and diseases such as NAFLD, fibrosis, and hepatocellular carcinoma.[29,30] As opposed to the hepatocytes, the cannabinoid signaling in hepatic nonparenchymal cells is relatively less explored. CB1R expression in HSCs was shown to have increased significantly in the rodent fibrosis model and cirrhotic human liver,[11,21] suggesting that endocannabinoids can act as pro-fibrogenic mediators in the liver. Moreover, the authors' previous studies have demonstrated that alcoholic steatosis is exacerbated through CB1R activation in hepatocytes by 2-AG produced from HSCs.[7,10] CB1R is also expressed in cholangiocytes, or bile duct epithelial cells, which are related to the pathophysiology of liver cirrhosis and primary biliary cirrhosis.[31] Furthermore, several studies have identified the close association of CB2R expressions in hepatic nonparenchymal cells and NAFLD progression, but detailed mechanisms have yet to be investigated. The distribution of the cannabinoid receptors in hepatic cells is briefly described in Figure 2.

Cannabinoid Signaling in the Pathogenesis of ALD

Alcohol Exposure and the Endocannabinoid System in ALD

Because alcohol exposure is considered a critical factor in causing complex physiological or pathological changes in the endocannabinoid system, curiosity about the biological function of cannabinoid receptors in ALD began to arise.[9,28] Consequently, the endocannabinoid system and its receptors were found to be involved in the pathophysiological mechanisms of ALD by regulating immune function, metabolic modulation, and

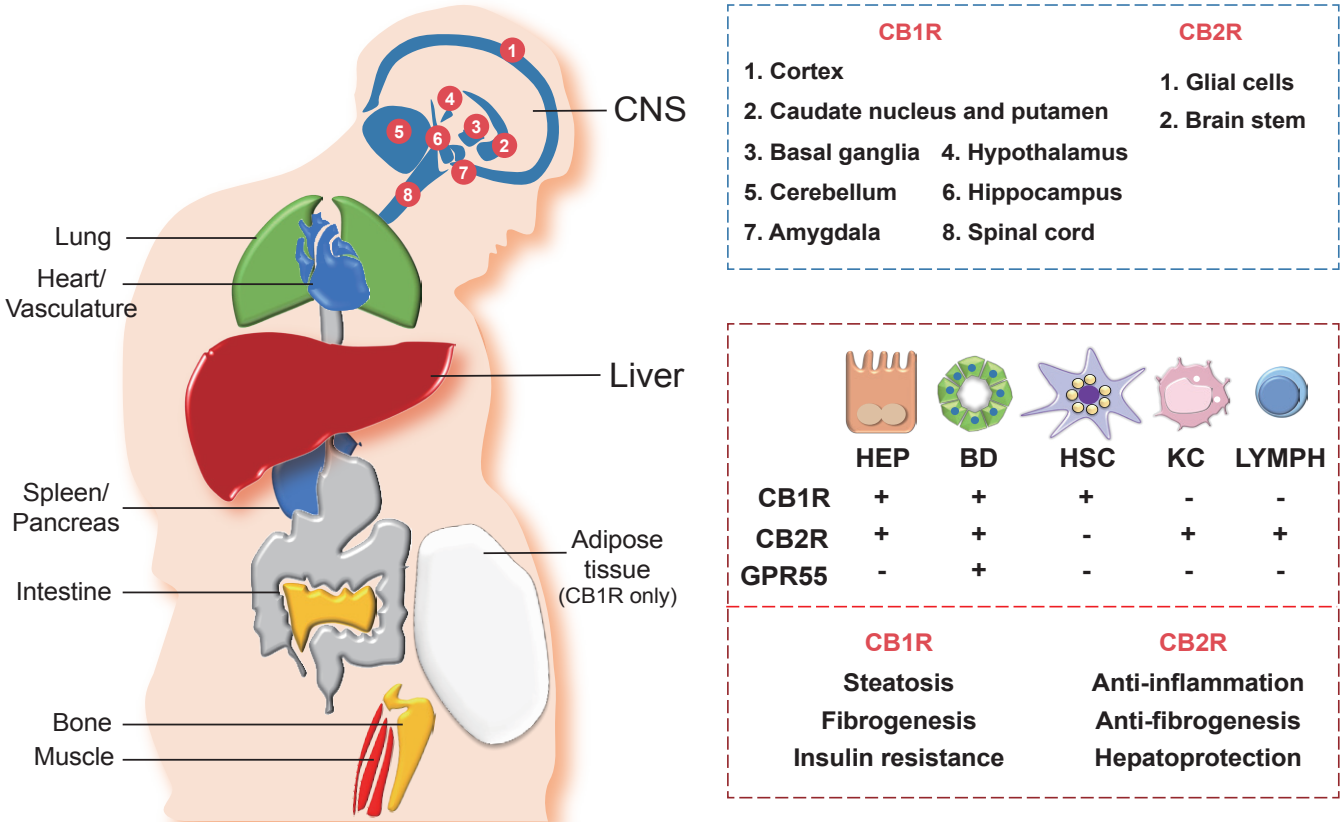

Figure 2. Distribution of cannabinoid receptors in various organs and hepatic cells. Cannabinoid receptors, cannabinoid-1 receptor (CB1R) and cannabinoid-2 receptor (CB2R), are expressed in various central and peripheral organs. CB1R and CB2R are most abundantly expressed in the central nervous system (CNS), where different parts of the CNS express either CB1R or CB2R (blue box). Both CB1R and CB2R are also expressed in peripheral organs including the heart, lung, spleen, pancreas, intestine, bone, muscle, and liver, as well as in the vascular system. Adipose tissues only express CB1R. In the liver, diverse types of cells—including hepatocytes (HEP), cholangiocytes (bile duct [BD] epithelial cells), hepatic stellate cells (HSC), Kupffer cells (KC), and lymphocytes (LYMPH)—differentially express cannabinoid receptors (CB1R and CB2R) and orphan G protein-coupled receptor 55 (GPR55), a noncannabinoid receptor that binds with endocannabinoids 2-AG and AEA (red box, top). Different functions of CB1R and CB2R in the liver are also indicated (red box, bottom).

inflammatory response in the onset and progression of ALD.[29,32] Because the expression of CB1R and CB2R is well identified in hepatocytes and various nonparenchymal cells in the liver, accurate comprehension of the regulatory mechanisms by which alcohol exposure generates or stimulates the production of endocannabinoids—as well as the effects of alcohol on the activation of cannabinoid receptors—could lead to a breakthrough in understanding the exact pathophysiology of ALD and in discovering potential therapeutic targets.

Alcoholic Liver Injury Through Cannabinoid Signaling

The pathological changes in the endocannabinoid system can lead to the development of several chronic liver diseases. Because the expressions of CB1R and CB2R increase in pathological conditions such as NAFLD, primary biliary cirrhosis, liver cirrhosis, and hepatocellular carcinoma, the hepatic endocannabinoid system is most likely to affect the onset of ALD.[9,28,29]

With the liver as the principal organ of alcohol metabolism, the majority of the alcohol consumed enters the liver to be metabolized, consequently activating the stress responses such as the production of ROS, inflammatory cytokines, or endoplasmic reticulum stress. These responses result in reduced fatty acid oxidation and enhanced hepatic lipogenesis.[6] Several animal experiments have established that chronic alcohol consumption could exacerbate alcoholic fatty liver by triggering abnormal CB1R-mediated signaling.[7,10] However, the authors' recent studies have clearly demonstrated that chronic alcohol consumption induces oxidative stress-mediated glutamate excretion from hepatocytes, which triggers the activation of mGluR5 to produce 2-AG, but not AEA, in HSCs via DAGL-beta. This, in turn, stimulates paracrine activation of hepatic CB1R,[7,10] which leads to the subsequent elevation of the expression of sterol regulatory element-binding protein-1c (SREBP1c), a representative lipogenic transcription factor located downstream of the CB1R signaling pathway.[7,30] As a result, the expression of target proteins of SREBP1c—namely acetyl coenzyme A (CoA) carboxylase and fatty acid synthase—are elevated, thereby inducing de novo lipogenesis in hepatocytes (see Figure 3).[23,33] This study served as a crucial opportunity to identify the involvement of the endocannabinoid system in metabolic regulation through bidirectional interaction between hepatocytes and HSCs in the liver. The fatty acids produced are then converted into triglyceride (TG), which should be excreted from the liver in the form of TG-rich very-low-density lipoprotein (VLDL). However, pharmacological blockade of CB1R (AM6545 and rimonabant) decreases the hepatocytes' ability to clear TG-rich VLDL, significantly reducing hepatic TG levels and markedly increasing the release of TG-rich VLDL in alcoholic and nonalcoholic fatty liver.[7,34]

In alcoholic liver injury and inflammation, the various types of ROS are one of the most important influential factors in the progression of ALD. The ROS is mainly generated through two metabolizing pathways that utilize different enzymes or proteins: alcohol dehydrogenase and cytochrome P450 2E1 (CYP2E1), which is a membrane protein that forms the cytochrome P450-dependent microsomal ethanol oxidizing system.[6] The importance of ROS in alcoholic liver injury has been portrayed in a study that reported the close relationship between the endocannabinoid system and ROS-induced liver injury in the pathophysiology of chronic alcohol consumption.[35] In this study, ethanol-induced 2-AG preferentially induced CB1R activation, followed by an upregulation in gene expression of estrogen-related receptor gamma (ERR-gamma), an orphan nuclear receptor. The authors explained that the increased expression of ERR-gamma enhances CYP2E1 induction, resulting in ROS-induced alcoholic liver injury. In addition, when ethanol was fed chronically to CB1R knockout mice, the expression of ERR-gamma and CYP2E1 decreased and alcoholic liver injury was significantly attenuated. Furthermore, administration of GSK5182, which is a selective inverse agonist of ERR-gamma, ameliorated alcoholic liver injury by reducing oxidative stress, confirming the criticality of cannabinoid receptor signaling in ROS-induced alcoholic liver injury.[35] Among the various inflammatory pathways activated in ALD, Kupffer cells, which are macrophages that reside in liver tissue, execute a crucial role in the onset of hepatic inflammation.[6] Currently, the most well-known mechanism of Kupffer cell activation is via lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) stimulation, by which the Kupffer cells acquire a pro-inflammatory phenotype.[6] Like other cells in the immune system, Kupffer cells mainly express CB2R rather than CB1R, and activation of CB2R exerts an anti-inflammatory property on Kupffer cells in the development of ALD.[29] In fact, when wild-type mice were fed with alcohol, Kupffer cells were polarized to the anti-inflammatory (M2) phenotype, whereas the pro-inflammatory (M1) phenotype was amplified in CB2R-deficient Kupffer cells in response to LPS stimulation.[36] In line with this observation, Kupffer cells also have been shown to acquire a protective property via the activation of their CB2R as regulated by an autophagy-dependent pathway, which further supports the essential role of CB2R in Kupffer cells.[37] Moreover, chronic alcohol consumption instigates the disruption of the intestinal epithelium, causing changes in gut permeability and increasing the level of LPS in the hepatic portal flow. Consequently, Kupffer cells become activated by TLR4. A study by Szabady et al. suggested a conceivable interplay between intestinal endocannabinoids and ALD. The authors demonstrated that intestinal endocannabinoids produced by epithelial cells could prevent inflammation and maintain homeostasis in a healthy gut by modulating neutrophil influx.[38] Thus, intestinal

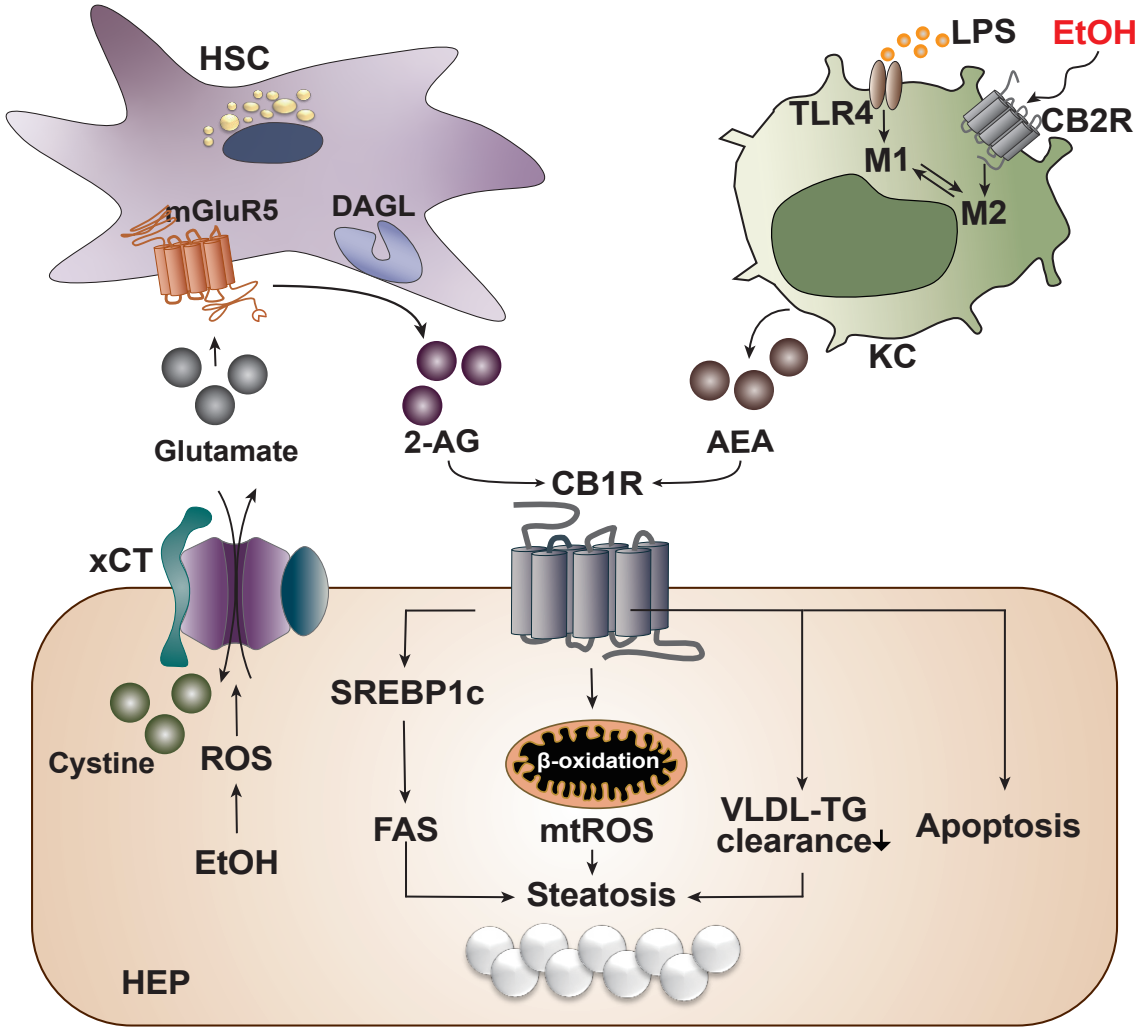

Figure 3. Cannabinoid signaling in the pathogenesis of alcohol-associated liver disease. Alcohol is mainly metabolized in hepatocytes (HEP) of the liver during which reactive oxygen species (ROS) is generated as a cellular stress response. The generated ROS stimulates and activates a cystine/glutamate antiporter (xCT) for the influx of cystine in exchange for the efflux of glutamate. The excreted glutamate then binds to a metabotropic glutamate receptor 5 (mGluR5) expressed in the neighboring hepatic stellate cells (HSC), inducing the production of 2-arachidonoyl glycerol (2-AG) by diacylglycerol lipase (DAGL). 2-AG produced in the HSC binds to cannabinoid-1 receptors (CB1R) expressed in the plasma membrane of neighboring HEP to induce de novo lipogenesis via the upregulation of sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthase (FAS). This forms a bidirectional paracrine loop pathway through which HEP and HSC in close proximity can metabolically regulate each other. Activation of CB1R can also induce β-oxidation of fatty acids in mitochondria, generating mitochondrial ROS (mtROS), which ultimately contributes to the accumulation of fat, or steatosis. Activated CB1R perturbs the excretion of triglyceride (TG) in the form of TG-rich very low-density lipoprotein (VLDL), further contributing to hepatic steatosis. CB1R activation is also known to induce apoptosis of cells. Kupffer cells (KC) normally become activated via the lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) stimulation and acquire a pro-inflammatory (M1) phenotype. However, when the CB2R expressed in Kupffer cells are stimulated by ethanol, they obtain an anti-inflammatory (M2) phenotype. Activated Kupffer cells then produce arachidonoyl ethanolamide (AEA), which also binds and activates CB1R in the neighboring HEP.

endocannabinoids might play beneficial roles in ALD-mediated gut leakage and the subsequent translocation of LPS to the liver.

CB1R also was found to modulate alcohol-induced liver fibrosis.[39] A study conducted by Patsenker et al. observed a strong expression of CB1R in the fibrotic septa of patients with alcohol-associated liver cirrhosis, and genetic and pharmacologic inhibition of CB1R attenuated both the hepatic inflammation and the alcoholic liver fibrosis by suppressing HSC activation.[39] Although it is well established that CB1R is involved in the development of hepatic steatosis and fibrosis, relatively few

studies have examined the role of CB2R in the pathophysiology of ALD. In a comparison study for the severity of hepatic steatosis, inflammation, and fibrosis using CB1R and CB2R knockout mice, the CB2R knockout mice showed severe fibrosis with aggravated steatosis and inflammation compared to those of the wild-type and CB1R knockout mice. This observation could be explained by the fact that the collagen production in activated HSCs was amplified in CB2R knockout mice,[40] indicating the protective role of CB2R in the progression of alcoholic liver fibrosis.

In brief, endocannabinoids have been found to have diverse effects on the pathophysiology of chronic liver disease, and various in vivo and in vitro experiments have been performed to investigate the characteristics of CB1R and CB2R in different types of ALD. To date, it is known that CB1R activation aggravates inflammation, steatosis, and fibrosis through the reduction of fatty acid oxidation and TG-VLDL secretion, enhanced de novo lipogenesis, and activation of HSCs, whereas CB2R inhibits inflammation and steatosis and has anti-fibrotic properties by exerting anti-inflammatory functions on Kupffer cells.[29,32] Figure 3 summarizes the opposite roles of CB1R and CB2R in the progression of ALD.

Glutamate-Mediated Endocannabinoid Production

As described earlier, one of the key mechanisms underlying the development of alcoholic fatty liver is the CB1R-mediated de novo lipogenesis in hepatocytes via the metabolic loop pathway.[7] However, questions remain as to which metabolic triggers lead to increased production of 2-AG in HSCs. Recently, the authors of this review substantiated that oxidative stress mediates the excretion of glutamate from the hepatocyte, stimulating the activation of mGluR5, which binds to glutamate, in nearby HSCs and leading to increased 2-AG production (see Figure 3).[10] Similar to other reports, this report also found that chronic alcohol consumption depleted antioxidant glutathione through the inhibition of the methionine cycle and the transsulfuration system, resulting in a shortage of cysteine. However, this study had a more striking discovery. First, the CYP2E1-mediated ROS production in hepatocytes significantly increased the xCT (cystine/glutamate antiporter)-mediated uptake of extracellular cystine, in exchange for the excretion of cytosolic glutamate, to compensate for the glutathione deficiency. Second, this parallel release of glutamate stimulated activation of mGluR5 in HSCs, which led to the production of 2-AG through mediation by DAGL-beta. As a result, the 2-AG produced activated CB1R in neighboring hepatocytes, inducing de novo lipogenesis. These findings suggest a bidirectional paracrine loop between hepatocytes and HSCs, named the "metabolic loop pathway," where both hepatocytes and HSCs regulate each other by either producing a neurotransmitter or expressing its receptor. Thus, the authors proposed a novel view of concept through this bidirectional signaling that utilizes a neurotransmitter, an endocannabinoid, and their respective receptors to operate at a metabolic synapse between hepatocytes and HSCs. In vivo experiments using genetic or pharmacologic inhibition of xCT or mGluR5 showed an improvement in alcohol-induced hepatic steatosis. More interestingly, plasma levels of glutamate were found to be elevated in ALD patients with hepatic steatosis and hepatitis but not in patients with fibrosis and cirrhosis, which suggests that the function of glutamate is not limited to the hepatic steatosis and further studies are strongly required to address this curiosity. In summary, the discovery of a bidirectional loop pathway between hepatocytes and HSCs suggested a new mechanism for the development of ALD, proposing the possibility of its application as a novel pharmacological target or an opportunity for glutamate as a prospective diagnostic marker in ALD.

Therapeutic Implications for ALD

Past and Current Pharmacological Approaches

Various animal experiments have established that hepatic endocannabinoids and their receptors play fundamental roles in the pathophysiology of chronic liver diseases, and pharmacological targeting of CB1R and CB2R for the treatment of liver diseases has been attempted.[29] Table 1 summarizes the effects of cannabinoid receptor–modulating drugs and their targets in animal models of ALD to date. Unfortunately, most clinical trials have been performed on patients with obesity, metabolic syndrome, and NAFLD, and only a few studies have explored and reported the beneficial efficacies of CB1R antagonists in the progression of hepatic steatosis, inflammation, and fibrosis.[21,25] In fact, clinical trials of cannabinoid receptor inhibitors have not been carried out in patients with ALD owing to the side effects of the drugs. For example, in a meta-analysis of nine clinical trials, adverse events, such as depression, anxiety, and nausea, were commonly observed with rimonabant at a dose of 20 mg per day for 6 to 24 months even though it had clinically meaningful results in metabolic disorders.[41]

Recently, a chemical compound that acts as a peripherally restricted antagonist of CB1R has been developed, which showed negligible CNS penetration and remarkable attenuation of alcoholic steatosis in mice.[42] Thus, there is a silver lining in the possibility that with refinement and adjustment, this chemical might be a profound lead compound that could undergo clinical trials as a novel therapeutic target. In short, a growing number of experimental findings on the involvement of hepatic endocannabinoids in the pathophysiology of ALD has enabled the development of endocannabinoid-based or cannabinoid

Table 1 Effects of Various Cannabinoid Receptor–Modulating Drugs and Their Target Cells in Different Animal Models of Alcohol-Associated Liver Disease, by Pharmacological Trial

Trial	Reagent	Receptor	Target Cell	Action	Research Model	Effect and Results
Jeong et al. (2008)[7]	Rimonabant	CB1R	Hepatocyte	Antagonist	Alcoholic fatty liver	Reduce steatosis (Lipogenesis↓, fatty acid oxidation↑)
Patsenker et al. (2016)[19]	Rimonabant	CB1R	HSC	Antagonist	In vitro experiment	Induce apoptosis Reduce pro-fibrotic property
Louvet et al. (2011)[36]	JWH-133	CB2R	Kupffer cell	Agonist	Alcoholic fatty liver	M2 polarization of Kupffer cell (Steatosis↓, inflammation↓)
Kim et al. (2013)[35]	GSK5182	ERR-gamma	Hepatocyte	Antagonist	Alcoholic fatty liver and inflammation	Reduce oxidative stress (CYP2E1 expression↓, hepatocyte apoptosis↓)
Amato et al. (2018)[42]	Compounds 25	CB1R	Hepatocyte	Antagonist	Alcoholic fatty liver	Peripherally restricted purine antagonist
Choi et al. (2019)[10]	CTEP	mGluR5	HSC	Antagonist	Alcoholic fatty liver	Inhibit mGluR5 and reduce steatosis (Lipogenesis↓, CB1R expression↓)
Choi et al. (2019)[10]	Sulfasalazine	xCT	Hepatocyte	Antagonist	Alcoholic fatty liver	Inhibit xCT and reduce steatosis (Lipogenesis↓, CB1R expression↓)

Note: The upward arrow (↑) indicates an increase, and the downward arrow (↓) indicates a decrease. CB1R, cannabinoid-1 receptor; CB2R, cannabinoid-2 receptor; CYP2E1, cytochrome P450 family 2 subfamily E member 1; ERR-gamma, estrogen-related receptor-gamma; HSC, hepatic stellate cell; mGluR5, metabotropic glutamate receptor 5; xCT, cystine/glutamate antiporter.

receptor–based pharmacological approaches that, it is hoped, could become a novel therapeutic strategy for ALD.

Limitation of the Current Cannabinoid-Based Treatment

Until now, there have been several clinical trials and reports in which a CB1R antagonist has been administered as treatment for obesity or metabolic risk factors.[43-45] The two most notable clinical trials are the ADAGIO-Lipids Trial and the Rimonabant in Obesity (RIO)-Europe study. In these clinical trials, cardiometabolic risk markers, such as body weight and lipid profiles, improved significantly when rimonabant, a well-known CB1R-selective antagonist, was administered to obese patients for 1 or 2 years, but the treatments were discontinued because of the psychiatric side effects including anxiety and depression.[45] Since then, the development of drugs with a mode of action restricted to the endocannabinoid system in the periphery has been undertaken. For example, peripheral organ-specific CB1R inverse agonist and antagonist (i.e., JD5037 and AM6545) were developed to reduce neuropsychiatric side effects, which were successful in reducing and improving cardiometabolic risks and hepatic steatosis in animal experiments.[34,46]

Apart from the CB1R antagonist, the pharmacological potential of the CB2R agonist, which is known to have hepatoprotective effects, also has been reevaluated.[36] Although only observed in mice, a study has confirmed that administration of JWH-133 (a CB2R agonist) exhibited improved alcoholic liver injury in mice by inducing the polarization of Kupffer cells into an M2 phenotype.[36,37] Interestingly, according to a recent cross-sectional study, cannabis users showed a significantly reduced prevalence of ALD of all spectrums (alcoholic steatosis, alcoholic steatohepatitis, alcohol-associated cirrhosis, and hepatocellular carcinoma). However, the underlying mechanism remains in question.[47] Based on the description above, one could speculate that the cannabis absorbed might activate CB2 receptors in immune cells or prevent intestinal leakage of endotoxins including LPS. Therefore, to date, no drugs targeting the endocannabinoid system are available for direct application to clinical trials in ALD patients, and further studies are required to study underlying mechanisms and to develop a treatment specifically effective for ALD.

Conclusions

Endocannabinoids are membranous lipid mediators that regulate diverse physiological functions in both the CNS and the peripheral organs, including the liver. Over the past 30 years, it has been found that the endocannabinoid system is involved in a variety of pathways associated with the onset, or the progression, of several diseases, including ALD. The endocannabinoid system has been observed in both the hepatocytes and various nonparenchymal cells in the liver, in which the endocannabinoid production and its receptor activation may contribute to the development of a spectrum of ALD, ranging from simple alcoholic steatosis to more severe forms such as steatohepatitis and fibrosis. Therefore, understanding the precise physiology of the endocannabinoid system in the liver and unveiling the mechanism underlying the association between ALD progression and hepatic endocannabinoid signaling seem to bear a paramount significance for the advancement of ALD treatment, as well as for the treatment of other chronic liver diseases (e.g., NAFLD, viral hepatitis). Moreover, developing efficacious and highly selective cannabinoid receptor–modulating drugs could be a major breakthrough in the treatment of ALD.

However, efforts to develop second- and third-generation CB1R antagonists must overcome the complications caused by the first generation of CB1R antagonists, which were able to penetrate the blood-brain barrier and produced critical psychiatric side effects. Furthermore, careful implication of the combinatorial effects of CB1R antagonist and CB2R agonist may bring about promising outcomes for the treatment of ALD in the future.

References

1. Witkiewitz K, Litten R, Leggio L. Advances in the science and treatment of alcohol use disorder. *Sci Adv.* 2019;5(9):eaax4043. https://doi.org/10.1126/sciadv.aax4043.
2. Grant BF, Chou SP, Saha TD, et al. Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001-2002 to 2012-2013: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *JAMA Psychiatry.* 2017;74(9):911-923. https://doi.org/10.1001/jamapsychiatry.2017.2161.
3. Xi B, Veeranki SP, Zhao M, Ma C, Yan Y, Mi J. Relationship of alcohol consumption to all-cause, cardiovascular, and cancer-related mortality in US adults. *J Am Coll Cardiol.* 2017;70(8):913-922. https://doi.org/10.1016/j.jacc.2017.06.054.
4. Stickel F, Datz C, Hampe J, Bataller R. Pathophysiology and management of alcoholic liver disease: Update 2016. *Gut Liver.* 2017;11(2):173-188. https://doi.org/10.5009/gnl16477.
5. Kim HH, Choi SE, Jeong WI. Oxidative stress and glutamate excretion in alcoholic steatosis: Metabolic synapse between hepatocyte and stellate cell. *Clin Mol Hepatol.* 2020;26(4):697-704. https://doi.org/10.3350/cmh.2020.0152.
6. Tilg H, Moschen AR, Kaneider NC. Pathways of liver injury in alcoholic liver disease. *J Hepatol.* 2011;55(5):1159-1161. https://doi.org/10.1016/j.jhep.2011.05.015.
7. Jeong WI, Osei-Hyiaman D, Park O, et al. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. *Cell Metab.* 2008;7(3):227-235. https://doi.org/10.1016/j.cmet.2007.12.007.
8. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol.* 1988;34(5):605-613.
9. Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G, Kunos G. Endocannabinoids in liver disease. *Hepatology.* 2011;53(1):346-355. https://doi.org/10.1002/hep.24077.
10. Choi WM, Kim HH, Kim MH, et al. Glutamate signaling in hepatic stellate cells drives alcoholic steatosis. *Cell Metab.* 2019;30(5):877-889.e7. https://doi.org/10.1016/j.cmet.2019.08.001.
11. Baldassarre M, Giannone FA, Napoli L, et al. The endocannabinoid system in advanced liver cirrhosis: Pathophysiological implication and future perspectives. *Liver Int.* 2013;33(9):1298-1308. https://doi.org/10.1111/liv.12263.
12. Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* 2006;58(3):389-462. https://doi.org/10.1124/pr.58.3.2.
13. Abood ME. Molecular biology of cannabinoid receptors. In: Pertwee RG, ed. *Cannabinoids. Handbook of Experimental Pharmacology.* Vol. 168. Berlin, Heidelberg, Germany: Springer; 2005:81-115. https://doi.org/10.1007/3-540-26573-2_3.
14. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov.* 2004;3(9):771-784. https://doi.org/10.1038/nrd1495.
15. Teixeira-Clerc F, Julien B, Grenard P, et al. CB1 cannabinoid receptor antagonism: A new strategy for the treatment of liver fibrosis. *Nat Med.* 2006;12(6):671-676. https://doi.org/10.1038/nm1421.
16. Julien B, Grenard P, Teixeira-Clerc F, et al. Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology.* 2005;128(3):742-755. https://doi.org/10.1053/j.gastro.2004.12.050.
17. Ashton JC, Rahman RM, Nair SM, Sutherland BA, Glass M, Appleton I. Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. *Neurosci Lett.* 2007;412(2):114-117. https://doi.org/10.1016/j.neulet.2006.10.053.
18. Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci.* 2003;4(11):873-884. https://doi.org/10.1002/cld.527.
19. Patsenker E, Stickel F. Cannabinoids in liver diseases. *Clin Liver Dis.* 2016;7(2):21-25. https://doi.org/10.1002/cld.527.
20. McKinney MK, Cravatt BF. Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem.* 2005;74:411-432. https://doi.org/10.1146/annurev.biochem.74.082803.133450.
21. Basu PP, Aloysius MM, Shah NJ, Brown NS Jr. Review article: The endocannabinoid system in liver disease, a potential therapeutic target. *Aliment Pharmacol Ther.* 2014;39(8):790-801. https://doi.org/10.1111/apt.12673.
22. Dinh T, Carpenter D, Leslie F, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci.* 2002;99(16):10819-10824. https://doi.org/10.1073/pnas.152334899.
23. Osei-Hyiaman D, DePetrillo M, Pacher P, et al. Endocannabinoid activation at hepatic CB 1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest.* 2005;115(5):1298-1305. https://doi.org/10.1172/jci200523057.
24. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. *Biochim Biophys Acta.* 2000;1535(1):78-86. https://doi.org/10.1016/s0925-4439(00)00085-5.
25. Reddy PM, Maurya N, Velmurugan BK. Medicinal use of synthetic cannabinoids—a mini review. *Curr Pharmacol Rep.* 2019;5(1):1-13. https://doi.org/10.1007/s40495-019-00181-w.

26. Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat.* 2002;68-69:619-631. https://doi.org/10.1016/s0090-6980(02)00060-6.
27. Buckley N. The peripheral cannabinoid receptor knockout mice: An update. *Br J Pharmacol.* 2008;153(2):309-318. https://doi.org/10.1038/sj.bjp.0707527.
28. Kunos G. Interactions between alcohol and the endocannabinoid system. *Alcohol Clin Exp Res.* 2020;44(4):790-805. https://doi.org/10.1111/acer.14306.
29. Mallat A, Teixeira-Clerc F, Lotersztajn S. Cannabinoid signaling and liver therapeutics. *J Hepatol.* 2013;59(4):891-896. https://doi.org/10.1016/j.jhep.2013.03.032.
30. Purohit V, Rapaka R, Shurtleff D. Role of cannabinoids in the development of fatty liver (steatosis). *AAPS J.* 2010;12(2):233-237. https://doi.org/10.1208/s12248-010-9178-0.
31. Floreani A, Lazzari R, Macchi V, et al. Hepatic expression of endocannabinoid receptors and their novel polymorphisms in primary biliary cirrhosis. *J Gastroenterol.* 2010;45(1):68-76. https://doi.org/10.1007/s00535-009-0122-y.
32. Lavanco G, Castelli V, Brancato A, Tringali G, Plescia F, Cannizzaro C. The endocannabinoid-alcohol crosstalk: Recent advances on a bi-faceted target. *Clin Exp Pharmacol Physiol.* 2018;45(9);889-896. https://doi.org/10.1111/1440-1681.12967.
33. Osei-Hyiaman D, Depetrillo M, Harvey-White J, et al. Cocaine-and amphetamine-related transcript is involved in the orexigenic effect of endogenous anandamide. *Neuroendocrinology.* 2005;81(4):273-282. https://doi.org/10.1159/000087925.
34. Tam J, Vemuri VK, Liu J, et al. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest.* 2010;120(8):2953-2966. https://doi.org/10.1172/jci42551.
35. Kim DK, Kim YH, Jang HH, et al. Estrogen-related receptor γ controls hepatic CB1 receptor-mediated CYP2E1 expression and oxidative liver injury by alcohol. *Gut.* 2013;62(7):1044-1054. https://doi.org/10.1136/gutjnl-2012-303347.
36. Louvet A, Teixeira-Clerc F, Chobert MN, et al. Cannabinoid CB2 receptors protect against alcoholic liver disease by regulating Kupffer cell polarization in mice. *Hepatology.* 2011;54(4):1217-1226. https://doi.org/10.1002/hep.24524.
37. Denaës T, Lodder J, Chobert MN, et al. The cannabinoid receptor 2 protects against alcoholic liver disease via a macrophage autophagy-dependent pathway. *Sci Rep.* 2016;6:28806. https://doi.org/10.1038/srep28806.
38. Szabady RL, Louissaint C, Lubben A, et al. Intestinal P-glycoprotein exports endocannabinoids to prevent inflammation and maintain homeostasis. *J Clin Invest.* 2018;128(9):4044-4056. https://doi.org/10.1172/jci96817.
39. Patsenker E, Stoll M, Millonig G, et al. Cannabinoid receptor type I modulates alcohol-induced liver fibrosis. *Mol Med.* 2011;17(11):1285-1294. https://doi.org/10.2119/molmed.2011.00149.
40. Trebicka J, Racz I, Siegmund SV, et al. Role of cannabinoid receptors in alcoholic hepatic injury: Steatosis and fibrogenesis are increased in CB2 receptor-deficient mice and decreased in CB1 receptor knockouts. *Liver Int.* 2011;31(6):860-870. https://doi.org/10.1111/j.1478-3231.2011.02496.x.
41. Sam AH, Salem V, Ghatei MA. Rimonabant: From RIO to ban. *J Obes.* 2011;2011:432607. https://doi.org/10.1155/2011/432607.
42. Amato GS, Manke A, Harris DL, et al. Blocking alcoholic steatosis in mice with a peripherally restricted purine antagonist of the type 1 cannabinoid receptor. *J Med Chem.* 2018;61(10):4370-4385. https://doi.org/10.1021/acs.jmedchem.7b01820.
43. Van Gaal LF, Scheen AJ, Rissanen AM, et al. Long-term effect of CB1 blockade with rimonabant on cardiometabolic risk factors: Two year results from the RIO-Europe Study. *Eur Heart J.* 2008;29(14):1761-1771. https://doi.org/10.1093/eurheartj/ehn076.
44. Després J-P, Ross R, Boka G, et al. Effect of rimonabant on the high-triglyceride/low–HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: The ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol.* 2009;29(3):416-423. https://doi.org/10.1161/atvbaha.108.176362.
45. Moreira FA, Crippa JAS. The psychiatric side-effects of rimonabant. *Braz J Psychiatry.* 2009;31(2):145-153. https://doi.org/10.1590/s1516-44462009000200012.
46. Tam J, Cinar R, Liu J, et al. Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metab.* 2012;16(2):167-179. https://doi.org/10.1016/j.cmet.2012.07.002.
47. Adejumo AC, Ajayi TO, Adegbala OM, et al. Cannabis use is associated with reduced prevalence of progressive stages of alcoholic liver disease. *Liver Int.* 2018;38(8):1475-1486. https://doi.org/10.1111/liv.13696.

ALCOHOL RESEARCH Current Reviews

Alcohol Res. 2021;41(1):11 | https://doi.org/10.35946/arcr.v41.1.11 Published: 9 September 2021

Alcohol and Cannabis Use and the Developing Brain

Briana Lees,[1] Jennifer Debenham,[1] and Lindsay M. Squeglia[2]

[1]Matilda Centre for Research in Mental Health and Substance Use, University of Sydney, Camperdown, Australia
[2]Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, South Carolina

Correspondence
Address correspondence concerning this article to Lindsay Squeglia, Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, 67 President Street, MSC 862, Charleston, SC 29425. Email: squegli@musc.edu

Acknowledgments
This article was supported by two grants from the National Institutes of Health: K23AA025399 and U01DA041093.

Disclosures
The authors declare no competing financial or nonfinancial interests.

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.

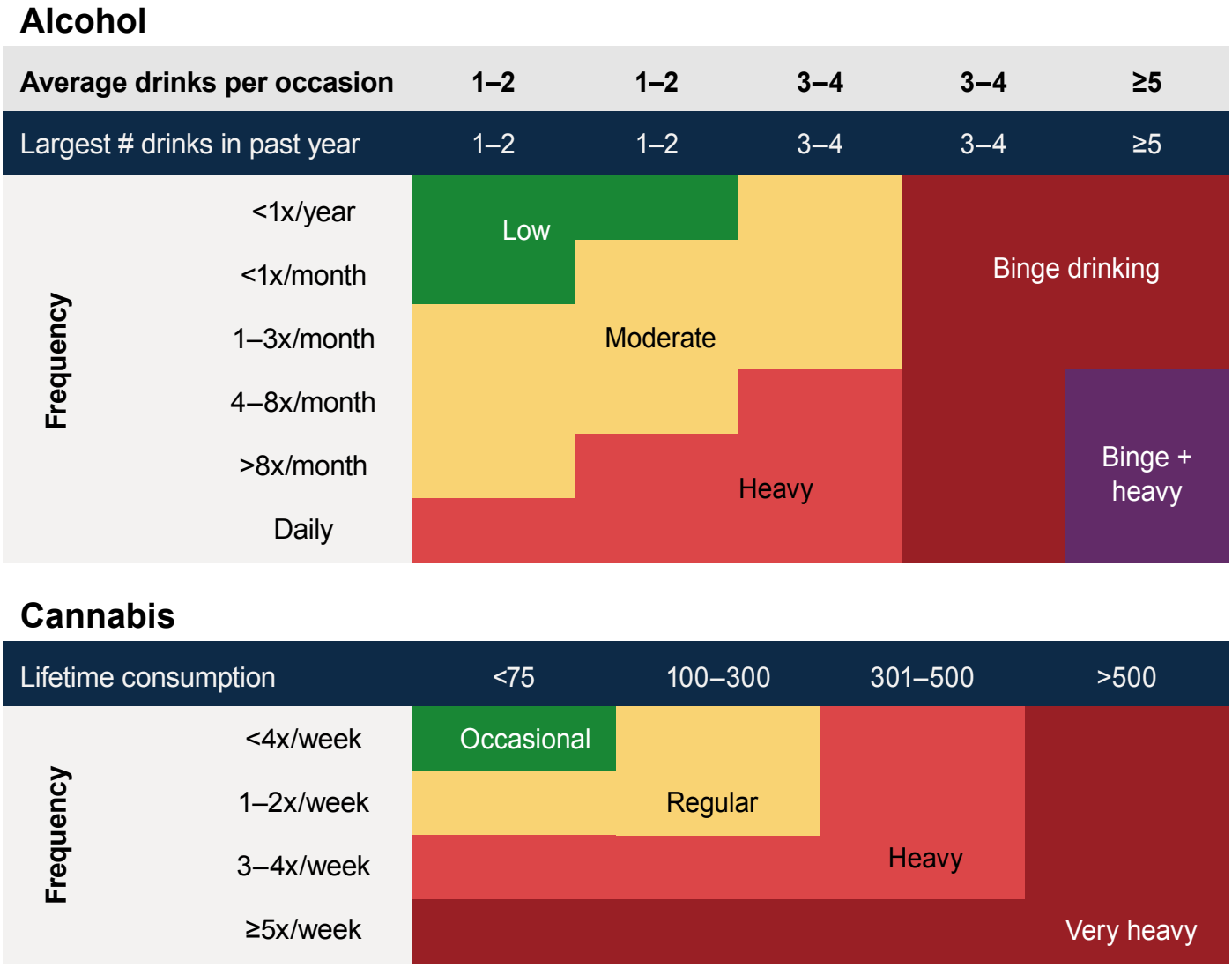

Figure 1. Typology of alcohol and cannabis use during adolescence. The charts are based on existing data classifying substance use groups during adolescence. Cannabis consumption is measured in occasions of cannabis use.[12,28]

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]

References

1. Sawyer SM, Azzopardi PS, Wickremarathne D, Patton GC. The age of adolescence. *Lancet Child Adolesc Health.* 2018;2(3):223-228. https://doi.org/10.1016/S2352-4642(18)30022-1.
2. Giedd JN. The teen brain: Insights from neuroimaging. *J Adolesc Health.* 2008;42(4):335-343. https://doi.org/10.1016/j.jadohealth.2008.01.007.
3. Giedd JN, Blumenthal J, Jeffries NO, et al. Brain development during childhood and adolescence: A longitudinal MRI study. *Nat Neurosci.* 1999;2(10):861-863. https://doi.org/10.1038/13158.
4. Pfefferbaum A, Mathalon DH, Sullivan EV, Rawles JM, Zipursky RB, Lim KO. A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Arch Neurol.* 1994;51(9):874-887. https://doi.org/10.1001/archneur.1994.00540210046012.
5. Gogtay N, Giedd JN, Lusk L, et al. Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci U S A.* 2004;101(21):8174-8179. https://doi.org/10.1073/pnas.0402680101.
6. Stiles J, Jernigan TL. The basics of brain development. *Neuropsychol Rev.* 2010;20(4):327-348. https://doi.org/10.1007/s11065-010-9148-4.
7. Raznahan A, Lee Y, Stidd R, et al. Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. *Proc Natl Acad Sci U S A.* 2010;107(39):16988-16993. https://doi.org/10.1073/pnas.1006025107.
8. Giorgio A, Watkins KE, Douaud G, et al. Changes in white matter microstructure during adolescence. *NeuroImage.* 2008;39(1):52-61. https://doi.org/10.1016/j.neuroimage.2007.07.043.
9. Casey BJ, Jones RM, Hare TA. The adolescent brain. *Ann N Y Acad Sci.* 2008;1124(1):111-126. https://doi.org/10.1196/annals.1440.010.
10. Steinberg L. A dual systems model of adolescent risk-taking. *Dev Psychobiol.* 2010;52(3):216-224. https://doi.org/10.1002/dev.20445.
11. Lees B, Garcia AM, Debenham J, Bryant BE, Mewton L, Squeglia LM. Promising vulnerability markers of substance use and misuse: A review of human neurobehavioral studies. *Neuropharmacology.* 2021;187:108500. https://doi.org/10.1016/j.neuropharm.2021.108500.
12. Lees B, Meredith LR, Kirkland AE, Bryant BE, Squeglia LM. Effect of alcohol use on the adolescent brain and behavior. *Pharmacol Biochem Behav.* 2020;192:172906. https://doi.org/10.1016/j.pbb.2020.172906.
13. Squeglia LM, Gray KM. Alcohol and drug use and the developing brain. *Curr Psychiatry Rep.* 2016;18(5):46. https://doi.org/10.1007/s11920-016-0689-y.
14. Mewton L, Lees B, Rao RT. Lifetime perspective on alcohol and brain health. *BMJ.* 2020;371(m4691). https://doi.org/10.1136/bmj.m4691.
15. Squeglia LM, Boissoneault J, Van Skike CE, Nixon SJ, Matthews DB. Age-related effects of alcohol from adolescent, adult, and aged populations using human and animal models. *Alcohol Clin Exp Res.* 2014;38(10):2509-2516. https://doi.org/10.1111/acer.12531.
16. Derefinko KJ, Charnigo RJ, Peters JR, Adams ZW, Milich R, Lynam DR. Substance use trajectories from early adolescence through the transition to college. *J Stud Alcohol Drugs.* 2016;77(6):924-935. https://doi.org/10.15288/jsad.2016.77.924.
17. World Health Organization (WHO). *Global Status Report on Alcohol and Health 2018*. WHO; 2018. https://apps.who.int/iris/bitstream/handle/10665/274603/9789241565639-eng.pdf.
18. Kokotailo PK. Alcohol use by youth and adolescents: A pediatric concern. *Pediatrics.* 2010;125(5):1078-1087. https://doi.org/10.1542/peds.2010-0438.
19. The United Nations Office on Drugs and Crime (UNODC). World Drug Report 2020. UNODC website. 2020. https://wdr.unodc.org/wdr2020/index2020.html.
20. Lees B, Mewton L, Stapinski LA, Squeglia LM, Rae CD, Teesson M. Neurobiological and cognitive profile of young binge drinkers: A systematic review and meta-analysis. *Neuropsychol Rev.* 2019;29(3):357-385. https://doi.org/10.1007/s11065-019-09411-w.
21. Hall W, Leung J, Lynskey M. The effects of cannabis use on the development of adolescents and young adults. *Ann Rev Dev Psychol.* 2020;2(1):461-483. https://doi.org/10.1146/annurev-devpsych-040320-084904.
22. Karoly HC, Ross JM, Ellingson JM, Feldstein Ewing SW. Exploring cannabis and alcohol co-use in adolescents: A narrative review of the evidence. *J Dual Diagn.* 2020;16(1):58-74. https://doi.org/10.1080/15504263.2019.1660020.
23. Carbia C, López-Caneda E, Corral M, Cadaveira F. A systematic review of neuropsychological studies involving young binge drinkers. *Neurosci Biobehav Rev.* 2018;90:332-349. https://doi.org/10.1016/j.neubiorev.2018.04.013.
24. Ewing SW, Sakhardande A, Blakemore S-J. The effect of alcohol consumption on the adolescent brain: A systematic review of MRI and fMRI studies of alcohol-using youth. *Neuroimage Clin.* 2014;5:420-437. https://doi.org/10.1016/j.nicl.2014.06.011.
25. Blest-Hopley G, Giampietro V, Bhattacharyya S. Regular cannabis use is associated with altered activation of central executive and default mode networks even after prolonged abstinence in adolescent users: Results from a complementary meta-analysis. *Neurosci Biobehav Rev.* 2019;96:45-55. https://doi.org/10.1016/j.neubiorev.2018.10.026.
26. Blest-Hopley G, Giampietro V, Bhattacharyya S. Residual effects of cannabis use in adolescent and adult brains—A meta-analysis of fMRI studies. *Neurosci Biobehav Rev.* 2018;88:26-41. https://doi.org/10.1016/j.neubiorev.2018.03.008.
27. Chye Y, Christensen E, Yücel M. Cannabis use in adolescence: A review of neuroimaging findings. *J Dual Diagn.* 2020;16(1):83-105. https://doi.org/10.1080/15504263.2019.1636171.
28. Debenham J, Birrell L, Champion K, Lees B, Yücel M, Newton NC. Examining neuropsychological and neurophysiological predictors and consequences of cannabis and illicit drug use during neurodevelopment: A systematic review of longitudinal studies in young people. *Lancet Child Adolesc Health.* 2021;5(8):589-604. https://doi.org/10.1016/s2352-4642(21)00051-1.
29. Schumann G, Loth E, Banaschewski T, et al. The IMAGEN study: Reinforcement-related behaviour in normal brain function and psychopathology. *Mol Psychiatry.* 2010;15(12):1128-1139. https://doi.org/10.1038/mp.2010.4.
30. Brown SA, Brumback T, Tomlinson K, et al. The National Consortium on Alcohol and NeuroDevelopment in Adolescence (NCANDA): A multisite study of adolescent development and substance use. *J Stud Alcohol Drugs.* 2015;76(6):895-908. https://doi.org/10.15288/jsad.2015.76.895.
31. Pfefferbaum A, Kwon D, Brumback T, et al. Altered brain developmental trajectories in adolescents after initiating drinking. *Am J Psychiatry.* 2018;175(4):370-380. https://doi.org/10.1176/appi.ajp.2017.17040469.
32. Sullivan EV, Brumback T, Tapert SF, et al. Disturbed cerebellar growth trajectories in adolescents who initiate alcohol drinking. *Biol Psychiatry.* 2020;87(7)632-644. https://doi.org/10.1016/j.biopsych.2019.08.026.
33. Squeglia LM, Tapert SF, Sullivan EV, et al. Brain development in heavy-drinking adolescents. *Am J Psychiatry.* 2015;172(6):531-542. https://doi.org/10.1176/appi.ajp.2015.14101249.
34. Luciana M, Collins PF, Muetzel RL, Lim KO. Effects of alcohol use initiation on brain structure in typically developing adolescents. *Am J Drug Alcohol Abuse.* 2013;39(6):345-355. https://doi.org/10.3109/00952990.2013.837057.
35. Jacobus J, Castro N, Squeglia LM, et al. Adolescent cortical thickness pre- and post-marijuana and alcohol initiation. *Neurotoxicol Teratol.* 2016;57:20-29. https://doi.org/10.1016/j.ntt.2016.09.005.

36. Robert GH, Luo Q, Yu T, et al. Association of gray matter and personality development with increased drunkenness frequency during adolescence. *JAMA Psychiatry.* 2020;77(4):409-419. https://doi.org/10.1001/jamapsychiatry.2019.4063.
37. Yu T, Jia T, Zhu L, et al. Cannabis-associated psychotic-like experiences are mediated by developmental changes in the parahippocampal gyrus. *J Am Acad Child Adolesc Psychiatry.* 2020;59(5):642-649. https://doi.org/10.1016/j.jaac.2019.05.034.
38. Jacobus J, Squeglia LM, Meruelo AD, et al. Cortical thickness in adolescent marijuana and alcohol users: A three-year prospective study from adolescence to young adulthood. *Dev Cogn Neurosci.* 2015;16:101-109. https://doi.org/10.1016/j.dcn.2015.04.006.
39. Jacobus J, Squeglia LM, Sorg SF, Nguyen-Louie TT, Tapert SF. Cortical thickness and neurocognition in adolescent marijuana and alcohol users following 28 days of monitored abstinence. *J Stud Alcohol Drugs.* 2014;75(5):729-743. https://doi.org/10.15288/jsad.2014.75.729.
40. O'Donnell LJ, Westin CF. An introduction to diffusion tensor image analysis. *Neurosurg Clin N Am.* 2011;22(2):185-196. https://doi.org/10.1016/j.nec.2010.12.004.
41. Zhao Q, Sullivan EV, Honnorat N, et al. Association of heavy drinking with deviant fiber tract development in frontal brain systems in adolescents. *JAMA Psychiatry.* 2021;78(4):407-415. https://doi.org/10.1001/jamapsychiatry.2020.4064.
42. Chavarria MC, Sánchez FJ, Chou YY, Thompson PM, Luders E. Puberty in the corpus callosum. *Neuroscience.* 2014;265:1-8. https://doi.org/10.1016/j.neuroscience.2014.01.030.
43. Jones SA, Kliamovich D, Nagel BJ. Sex hormones partially explain the sex-dependent effect of lifetime alcohol use on adolescent white matter microstructure. *Psychiatry Res Neuroimaging.* 2021;307:111230. https://doi.org/10.1016/j.pscychresns.2020.111230.
44. Bava S, Jacobus J, Thayer RE, Tapert SF. Longitudinal changes in white matter integrity among adolescent substance users. *Alcohol Clin Exp Res.* 2013;37(suppl 1):E181-E189. https://doi.org/10.1111/j.1530-0277.2012.01920.x.
45. Jacobus J, Squeglia LM, Bava S, Tapert SF. White matter characterization of adolescent binge drinking with and without co-occurring marijuana use: A 3-year investigation. *Psychiatry Res.* 2013;214(3):374-381. https://doi.org/10.1016/j.pscychresns.2013.07.014.
46. Jurk S, Mennigen E, Goschke T, Smolka MN. Low-level alcohol consumption during adolescence and its impact on cognitive control development. *Addict Biol.* 2018;23(1):313-326. https://doi.org/10.1111/adb.12467.
47. Zhao Q, Sullivan EV, Müller-Oehring EM, et al. Adolescent alcohol use disrupts functional neurodevelopment in sensation seeking girls. *Addict Biol.* 2021;26(2):e12914. https://doi.org/10.1111/adb.12914.
48. Spechler PA, Chaarani B, Orr C, et al. Longitudinal associations between amygdala reactivity and cannabis use in a large sample of adolescents. *Psychopharmacology (Berl).* 2020;237(11):3447-3458. https://doi.org/10.1007/s00213-020-05624-7.
49. Tervo-Clemmens B, Simmonds D, Calabro FJ, et al. Early cannabis use and neurocognitive risk: A prospective functional neuroimaging study. *Biol Psychiatry Cogn Neurosci Neuroimaging.* 2018;3(8):713-725. https://doi.org/10.1016/j.bpsc.2018.05.004.
50. Camchong J, Lim KO, Kumra S. Adverse effects of cannabis on adolescent brain development: A longitudinal study. *Cereb Cortex.* 2017;27(3):1922-1930. https://doi.org/10.1093/cercor/bhw015.
51. Pujol J, Blanco-Hinojo L, Batalla A, et al. Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users. *J Psychiatr Res.* 2014;51:68-78. https://doi.org/10.1016/j.jpsychires.2013.12.008.
52. Kim-Spoon J, Herd T, Brieant A, et al. Bidirectional links between adolescent brain function and substance use moderated by cognitive control. *J Child Psychol Psychiatry.* 2021;62(4):427-436. https://doi.org/10.1111/jcpp.13285.
53. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD. The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: A latent variable analysis. *Cogn Psychol.* 2000;41(1):49-100. https://doi.org/10.1006/cogp.1999.0734.
54. Miyake A, Friedman NP. The nature and organization of individual differences in executive functions: Four general conclusions. *Curr Dir Psychol Sci.* 2012;21(1):8-14. https://doi.org/10.1177/0963721411429458.
55. Diamond A. Executive functions. *Annu Rev Psychol.* 2013;64:135-168. https://doi.org/10.1146/annurev-psych-113011-143750.
56. Boelema SR, Harakeh Z, van Zandvoort MJE, et al. Adolescent heavy drinking does not affect maturation of basic executive functioning: Longitudinal findings from the TRAILS study. *PLoS One.* 2015;10(10):e0139186-e0139186. https://doi.org/10.1371/journal.pone.0139186.
57. Carbia C, Cadaveira F, Caamaño-Isorna F, Rodríguez Holguín S, Corral M. Binge drinking trajectory and decision-making during late adolescence: Gender and developmental differences. *Front Psychol.* 2017;8:783. https://doi.org/10.3389/fpsyg.2017.00783.
58. Nguyen-Louie TT, Castro N, Matt GE, Squeglia LM, Brumback T, Tapert SF. Effects of emerging alcohol and marijuana use behaviors on adolescents' neuropsychological functioning over four years. *J Stud Alcohol Drugs.* 2015;76(5):738-748. https://doi.org/10.15288/jsad.2015.76.738.
59. Hendriks H, van de Rest O, Snippe A, Kieboom J, Hogenelst K. Alcohol consumption, drinking patterns, and cognitive performance in young adults: A cross-sectional and longitudinal analysis. *Nutrients.* 2020;12(1):200. https://doi.org/10.3390/nu12010200.
60. Mota N, Parada M, Crego A, et al. Binge drinking trajectory and neuropsychological functioning among university students: A longitudinal study. *Drug Alcohol Depend.* 2013;133:108-114. https://doi.org/10.1016/j.drugalcdep.2013.05.024.
61. Noorbakhsh S, Afzali MH, Boers E, Conrod PJ. Cognitive function impairments linked to alcohol and cannabis use during adolescence: A study of gender differences. *Front Hum Neurosci.* 2020;14:95. https://doi.org/10.3389/fnhum.2020.00095.
62. Meier MH, Caspi A, Ambler A, et al. Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proc Natl Acad Sci U S A.* 2012;109(40):E2657-E2664. https://doi.org/10.1073/pnas.1206820109.
63. Infante MA, Nguyen-Louie TT, Worley M, Courtney KE, Coronado C, Jacobus J. Neuropsychological trajectories associated with adolescent alcohol and cannabis use: A prospective 14-year study. *J Int Neuropsychol Soc.* 2020;26(5):480-491. https://doi.org/10.1017/S1355617719001395.
64. Becker MP, Collins PF, Schultz A, Urošević S, Schmaling B, Luciana M. Longitudinal changes in cognition in young adult cannabis users. *J Clin Exp Neuropsychol.* 2018;40(6):529-543. https://doi.org/10.1080/13803395.2017.1385729.
65. Ivanov I, Parvaz MA, Velthorst E, et al. Substance use initiation, particularly alcohol, in drug-naive adolescents: Possible predictors and consequences from a large cohort naturalistic study. *J Am Acad Child Adolesc Psychiatry.* 2021;60(5):623-636. https://doi.org/10.1016/j.jaac.2020.08.443.
66. Jones SA, Steele JS, Nagel BJ. Binge drinking and family history of alcoholism are associated with an altered developmental trajectory of impulsive choice across adolescence. *Addiction.* 2017;112(7):1184-1192. https://doi.org/10.1111/add.13823.
67. Squeglia LM, Spadoni AD, Infante MA, Myers MG, Tapert SF. Initiating moderate to heavy alcohol use predicts changes in neuropsychological functioning for adolescent girls and boys. *Psychol Addict Behav.* 2009;23(4):715-722. https://doi.org/10.1037/a0016516.
68. Wallace AL, Wade NE, Lisdahl KM. Impact of 2 weeks of monitored abstinence on cognition in adolescent and young adult cannabis users. *J Int Neuropsychol Soc.* 2020;26(8):776-784. https://doi.org/10.1017/S1355617720000260.

69. Tapert SF, Granholm E, Leedy NG, Brown SA. Substance use and withdrawal: Neuropsychological functioning over 8 years in youth. *J Int Neuropsychol Soc.* 2002;8(7):873-883. https://doi.org/10.1017/s1355617702870011.
70. Nguyen-Louie TT, Tracas A, Squeglia LM, Matt GE, Eberson-Shumate S, Tapert SF. Learning and memory in adolescent moderate, binge, and extreme-binge drinkers. *Alcohol Clin Exp Res.* 2016;40(9):1895-1904. https://doi.org/10.1111/acer.13160.
71. Carbia C, Cadaveira F, Caamano-Isorna F, Rodriguez-Holguin S, Corral M. Binge drinking during adolescence and young adulthood is associated with deficits in verbal episodic memory. *PLoS One.* 2017;12(2):e0171393. https://doi.org/10.1371/journal.pone.0171393.
72. Barthelemy OJ, Richardson MA, Heeren TC, et al. Do differences in learning performance precede or follow initiation of marijuana use? *J Stud Alcohol Drugs.* 2019;80(1):5-14. https://doi.org/10.15288/jsad.2019.80.5.
73. Tait RJ, Mackinnon A, Christensen H. Cannabis use and cognitive function: 8-year trajectory in a young adult cohort. *Addiction.* 2011;106(12):2195-2203. https://doi.org/10.1111/j.1360-0443.2011.03574.x.
74. Hanson KL, Cummins K, Tapert SF, Brown SA. Changes in neuropsychological functioning over 10 years following adolescent substance abuse treatment. *Psychol Addict Behav.* 2011;25(1):127-142. https://doi.org/10.1037/a0022350.
75. Hanson KL, Medina KL, Padula CB, Tapert SF, Brown SA. Impact of adolescent alcohol and drug use on neuropsychological functioning in young adulthood: 10-year outcomes. *J Child Adolesc Subst Abuse.* 2011;20(2):135-154. https://doi.org/10.1080/1067828x.2011.555272.
76. Jackson NJ, Isen JD, Khoddam R, et al. Impact of adolescent marijuana use on intelligence: Results from two longitudinal twin studies. *Proc Natl Acad Sci U S A.* 2016;113(5):E500-E508. https://doi.org/10.1073/pnas.1516648113.
77. Meier MH, Caspi A, Danese A, et al. Associations between adolescent cannabis use and neuropsychological decline: A longitudinal co-twin control study. *Addiction.* 2018;113(2):257-265. https://doi.org/10.1111/add.13946.
78. Fried P, Watkinson B, James D, Gray R. Current and former marijuana use: Preliminary findings of a longitudinal study of effects on IQ in young adults. *CMAJ.* 2002;166(7):887-891.
79. Han B, Compton WM, Blanco C, Crane E, Lee J, Jones CM. Prescription opioid use, misuse, and use disorders in U.S. adults: 2015 National Survey on Drug Use and Health. *Ann Intern Med.* 2017;167(5):293-301. https://doi.org/10.7326/M17-0865.
80. Fadus MC, Smith TT, Squeglia LM. The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. *Drug Alcohol Depend.* 2019;201:85-93. https://doi.org/10.1016/j.drugalcdep.2019.04.011.
81. Jordan CJ, Andersen SL. Sensitive periods of substance abuse: Early risk for the transition to dependence. *Dev Cogn Neurosci.* 2017;25:29-44. https://doi.org/10.1016/j.dcn.2016.10.004.
82. Lees B, Mewton L, Jacobus J, et al. Association of prenatal alcohol exposure with psychological, behavioral, and neurodevelopmental outcomes in children from the adolescent brain cognitive development study. *Am J Psychiatry.* 2020;177(11). https://doi.org/10.1176/appi.ajp.2020.20010086.
83. Marinescu IE, Lawlor PN, Kording KP. Quasi-experimental causality in neuroscience and behavioural research. *Nat Hum Behav.* 2018;2(12):891-898. https://doi.org/10.1038/s41562-018-0466-5.
84. Tomko RL, Gray KM, Oppenheimer SR, Wahlquist AE, McClure EA. Using REDCap for ambulatory assessment: Implementation in a clinical trial for smoking cessation to augment in-person data collection. *Am J Drug Alcohol Abuse.* 2019;45(1):26-41. https://doi.org/10.1080/00952990.2018.1437445.
85. Tomko RL, McClure EA, Squeglia LM, et al. Methods to reduce the incidence of false negative trial results in substance use treatment research. *Curr Opin Psychol.* 2019;30:35-41. https://doi.org/10.1016/j.copsyc.2019.01.009.
86. ElSohly MA, Mehmedic Z, Foster S, Gon C, Chandra S, Church JC. Changes in cannabis potency over the last 2 decades (1995–2014): Analysis of current data in the United States. *Biol Psychiatry.* 2016;79(7):613-619. https://doi.org/10.1016/j.biopsych.2016.01.004.
87. Tomko RL, Baker NL, McClure EA, et al. Incremental validity of estimated cannabis grams as a predictor of problems and cannabinoid biomarkers: Evidence from a clinical trial. *Drug Alcohol Depend.* 2018;182:1-7. https://doi.org/10.1016/j.drugalcdep.2017.09.035.
88. National Institutes of Health. Notice of information: Establishment of a standard THC unit to be used in research. 2021. https://grants.nih.gov/grants/guide/notice-files/NOT-DA-21-049.html.
89. Lisdahl KM, Sher KJ, Conway KP, et al. Adolescent Brain Cognitive Development (ABCD) Study: Overview of substance use assessment methods. *Dev Cogn Neurosci.* 2018;32:80-96. https://doi.org/10.1016/j.dcn.2018.02.007.
90. Debenham J, Birrell L, Champion K, Askovic M, Newton N. A pilot study of a neuroscience-based, harm minimisation programme in schools and youth centres in Australia. *BMJ Open.* 2020;10(2):e033337. https://doi.org/10.1136/bmjopen-2019-033337.
91. Meredith LR, Maralit AM, Thomas SE, et al. Piloting of the Just Say Know prevention program: A psychoeducational approach to translating the neuroscience of addiction to youth. *Am J Drug Alcohol Abuse.* 2021;47(1):16-25. https://doi.org/10.1080/00952990.2020.1770777.
92. Silvers JA, Squeglia LM, Rømer Thomsen K, Hudson KA, Feldstein Ewing SW. Hunting for what works: Adolescents in addiction treatment. *Alcohol Clin Exp Res.* 2019;43(4):578-592. https://doi.org/10.1111/acer.13984.