THE ENDOCANNABINOID SYSTEM: FILLING THE TRANSLATIONAL GAP BETWEEN NEUROSCIENCE AND PSYCHIATRY

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THE ENDOCANNABINOID SYSTEM: FILLING THE TRANSLATIONAL GAP BETWEEN NEUROSCIENCE AND PSYCHIATRY

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EDITORIAL
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Editorial: The Endocannabinoid System: Filling the Translational Gap Between Neuroscience and Psychiatry

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Editorial on the Research Topic

The Endocannabinoid System: Filling the Translational Gap Between Neuroscience and Psychiatry

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Translational research has become a priority in every branch of medicine. In psychiatry, it has taken on the goal of advancing our understanding of the neurobiological underpinnings of cognition, behavior, and emotion, and the pathophysiological mechanisms affecting such processes that lead to the development of mental disorders. If successful in identifying objective measures of psychopathology and biomarkers of disease states, translational research will have a tremendous impact on psychiatry, allowing the achievement of a biologically relevant nosology and the development of treatments based on individual characteristics, thus giving rise to the era of personalized treatments (1).

Widely used worldwide, cannabinoids have attracted much attention for their potential role in health and disease, especially in psychiatry (2). Translational research has significantly advanced our understanding of the neuropsychiatric effects of cannabinoids (3–6). Adopting a translational perspective, the Research Topic presented here brings together up-to-date knowledge of these fascinating and complex chemical substances and how they may modulate mental health.

A recent report from the National Institute on Drug Abuse (NIDA) drew attention to the risks that using tobacco, alcohol, or illicit drugs, and misusing prescribed medication during pregnancy can carry for the offspring (https://www.drugabuse.gov/download/18910/substance-use-in-women-research-report.pdf?v=b802679e27577e5e5365092466ac42e8). Such warning stems from the evidence that substances can pass easily through the placenta to reach the fetus. In their review, Navarrete et al. summarize the evidence regarding the specific effects of cannabis use during the prenatal and postnatal periods, indicating behavioral and neurobiological aberrancies that can potentially persist throughout childhood and adolescence, and increased risk for the development of psychiatric disorders later in life, especially affective and substance use disorders (SUD). In their review, Nashed et al. come to similar conclusions, highlighting the risks specifically associated

with consumption of cannabis varieties with high content of delta-9-tetrahydrocannabinol (Δ-9-THC), the main psychoactive exogenous cannabinoid, that is believed to cross the placenta and impact development. High use of cannabis during pregnancy, underestimation of its risk, progressive increase in cannabis potency in terms of Δ-9-THC concentration over the last two decades, and long-lasting neurodevelopmental consequences have been reported. Such evidence made authors emphasize the need to accelerate our knowledge of the effects of cannabis exposure during pregnancy and lactation across the life span.

One potential explanation for the higher risk of SUD during adolescence and adulthood, when prenatally exposed to cannabis, is that exogenous cannabinoids may affect the neurodevelopment of the endocannabinoid system (ECS), disrupting neurotransmission in brain areas regulating reward and motivation, thus increasing vulnerability to subsequent substance use and addiction. In their gene-by-gene interaction study, Elkrief et al. find genetically determined susceptibility to problem drinking related to specific polymorphisms in the *CNR1* gene, the gene coding for the cannabinoid receptor type 1 (CB1) protein, and other genes involved in endocannabinoid metabolism. Altogether, evidence points in the direction of alterations of the ECS, both genetically determined and environmentally induced, in the development of addictive behaviors. Of further interest, Pallanti et al. discuss the hypothesis that the ECS is crucial not only to *substance* addiction but also to *behavioral* addiction such as gambling disorder, now counted in the DSM-5 "substance-related and addictive disorders" section to highlight common biobehavioral underpinnings with SUD. To date, there is interest in the possibility that cannabidiol (CBD), the most studied compound in cannabis after Δ-9-THC, may modulate reward, decisional and sensorimotor processes, being a viable treatment for behavioral addictions.

However, effects of cannabinoids on mental health are far from straightforward. A wide range of effects have been reported over the last decades, from accelerating disease processes to potentially halting them. Graczyk et al. offer an overview of these effects, indicating treatment potential for anxiety, mood, sleep disorders, and addiction, as well as detrimental consequences in terms of psychosis risk and cognitive impairments. Authors link such apparent contrasting evidence to differential effects of cannabis depending on ECS activity, phytocannabinoid composition (Δ-9-THC vs. CBD), terpenoid composition, and dose. Research data from McPherson et al. also indicate an important role of sex in driving the effects of cannabinoids on mental health. More specifically, chronic cannabis use is found to be associated with smaller cerebellum volume and poorer sleep quality, the latter being more pronounced in early-onset cannabis users. Interestingly, females were more sensitive than males to such effects of chronic cannabis use.

Recent years have witnessed a growing interest in the possibility of targeting the ECS to treat major psychiatric disorders. Articles published in this Research Topic are no exception. Cheung et al. address the role of the ECS in neurodevelopment, reviewing the evidence that early cannabinoid treatment may be beneficial under severe conditions such as autism spectrum disorder. To date, CBD has shown the most promising results, also showing a satisfactory safety profile. Also, Cortez et al. review the evidence in support of targeting the ECS in psychosis, beyond the use of antipsychotics aimed at correcting the hyperdopaminergic state seen in the disorder. The authors propose that the cannabinoid receptor type 2 (CB2) may be relevant to different pathophysiological processes observed in psychosis, including not only modulation of dopaminergic neurotransmission, but also microglial activation and stress-induced neuroplastic changes. Again, CBD seems to be a valid treatment also for psychosis, as suggested by Hoffman in his review of preclinical evidence corroborating ECS aberrancies in this disorder and different modulatory effects of CBD on ECS function. Further, Thippaiah et al. discuss the evidence of ECS dysfunction in the context of depression and suicidal behavior, possibly by modulating the hypothalamic-pituitary-adrenal (HPA) axis, neurotrophic factor such as brain derived neurotrophic factor (BDNF), and other neurotransmitters including serotonin, norepinephrine, and dopamine. In his opinion article, Pinna suggests that better characterizing the role of the ECS in mood disorders and comorbid suicidal behavior may result in the identification of more precise neurobiological targets and the related development of novel pharmacological treatments for these conditions.

Finally, Kayser et al. explore in their methods article the translational potential and limitations of human laboratory studies of the effects of cannabinoids. Such studies, especially when implemented with imaging and other neurobiological measures, have helped in modeling addiction, studying the role of cannabinoids in psychiatric disorders, and investigating treatment options. Limited generalizability and participant selection are the main Achilles' heel in these studies. The authors clearly tip the balance in favor of laboratory models as they represent a key translational bridge to inform which preclinical evidence has a chance to result in a successful large-scale clinical study and, possibly, bring a new molecule to the market.

The last decades have seen progressively diminishing numbers of novel drugs between the preclinical and clinical stages of development (7). Translational research must continue to evolve in response to the need to reverse course. Determining and improving the predictive validity of both animal and human laboratory models is one of the challenges of the near future (8). Our hope of developing new medications for psychiatric disorders depends on it.

AUTHOR CONTRIBUTIONS

All authors have contributed to the editorial and critically reflected on the successive versions.

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REVIEW
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Are CB2 Receptors a New Target for Schizophrenia Treatment?

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Schizophrenia is a complex disorder that involves several neurotransmitters such as dopamine, glutamate, and GABA. More recently, the endocannabinoid system has also been associated with this disorder. Although initially described as present mostly in the periphery, cannabinoid type-2 (CB2) receptors are now proposed to play a role in several brain processes related to schizophrenia, such as modulation of dopaminergic neurotransmission, microglial activation, and neuroplastic changes induced by stress. Here, we reviewed studies describing the involvement of the CB2 receptor in these processes and their association with the pathophysiology of schizophrenia. Taken together, these pieces of evidence indicate that CB2 receptor may emerge as a new target for the development of antipsychotic drugs.

Keywords: cannabinoids, endocannabinoid system, psychosis, dopamine, microglia

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INTRODUCTION

Schizophrenia is a highly disabling psychiatric disorder of multifactorial etiology that affects about 1% of the world population (1). The symptoms of schizophrenia are divided into three main groups: positive, negative, and cognitive symptoms. Positive symptoms are characterized by an exaggeration of normal functions, presenting mainly as hallucinations, delusional ideas, defragmentation of thought, and psychomotor agitation. On the other hand, the negative symptoms are characterized by a loss of normal functions, leading to affective blunting, anhedonia, and social withdrawal (2). The cognitive symptoms are related to deficits in domains such as working memory, attention, verbal learning and memory, problem-solving, among others (3).

Although the pathophysiology of schizophrenia remains mostly unknown, it has long been thought that it involves an imbalance among several neurotransmitter systems. The first, and likely the most influential, hypothesis about the neurobiology of schizophrenia proposes that changes in the dopamine system, mainly a striatal hyperdopaminergic state, would be responsible for the psychotic symptoms (4). Following this initial proposal, it was later suggested that negative and cognitive symptoms would be associated with a hypodopaminergic state in the prefrontal cortex (PFC) (5).

The first drugs used to treat schizophrenia, known as typical antipsychotics, act as antagonists at dopamine D2 receptors. Besides their effects on positive symptoms, they also cause adverse effects such as extrapyramidal side effects and hyperprolactinemia, resulting in a high discontinuation rate. The second-generation or atypical antipsychotics, despite also targeting dopamine D2 receptors, also bind to receptors associated with other neurotransmitter systems (6). Although these drugs have a lower tendency to induce adverse motor effects at therapeutic doses than first-generation antipsychotics, they are associated with undesirable effects that may limit their use, such as metabolic changes and weight gain (7, 8). In addition, while positive symptoms have a good clinical

response to typical and atypical antipsychotics, the negative and cognitive impairments are more resistant to the available drugs. Together, these observations support the urgent need to develop new drugs with better efficacy and tolerability (9–11).

Considering the lack of therapeutic options and the complexity of this disorder, recent hypotheses have emerged involving other neurotransmitter systems such as the glutamatergic, serotonergic, gamma-aminobutyric acid (GABA), and, more recently, the endocannabinoid (12–16).

THE ENDOCANNABINOID SYSTEM

The endocannabinoid system (ECS) is a modulatory system that plays a crucial role in brain development, synaptic plasticity, and response to endogenous and environmental insults (17). The ECS comprises endogenous cannabinoids (endocannabinoids), cannabinoid receptors, and the enzymes responsible for the synthesis and degradation of endocannabinoids. The two main and best-characterized endocannabinoids are N-arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG) which, unlike most classical neurotransmitters, are produced on demand. There are reports, however, indicating that they might also be stored intracellularly (18, 19).

In the central nervous system (CNS) anandamide and 2-AG are synthesized and secreted from postsynaptic neurons. They bind to cannabinoid CB1 and CB2 receptors located on presynaptic terminals, acting as retrograde messengers and to CB2 receptors located on the postsynaptic site of some neurons (20). Once released in the synaptic cleft, endocannabinoids can be taken up by specific transport proteins and then broken down by the fatty acid amid hydrolase (FAAH) and monoacylglycerol lipase (MAGL) enzymes, which degrades anandamide and 2-AG, respectively (21, 22).

Although the effects of endocannabinoids are mediated mainly by CB1 and CB2 cannabinoid receptors, others receptors such as the peroxisome proliferator-activated receptors (PPARs) and transient receptor potential (TRP) channels, can also be activated by these compounds (17, 23). CB1 and CB2 receptors are G-protein-coupled receptors (GPCRs) that, in addition to interacting with endocannabinoids, are also activated by synthetic and plant-derived cannabinoids.

The cannabinoid receptors are G protein-coupled receptors (GPCR), which couple mainly to the G_i and G_0 classes of G proteins. Their activation inhibits the adenylyl cyclases enzymes, activates mitogen-activated protein kinases and modulates voltage-dependent ion channels (i.e., activating voltage-dependent potassium channels and inhibiting voltage-dependent calcium channels) (23). Overall, the intracellular signaling induced by the activation of cannabinoid receptors inhibits neurotransmitter release (17).

CB1 receptors are the most prevalent GPCR in the CNS and are located mainly in the cortex, hippocampus, amygdala, basal ganglia, and cerebellum (24). This receptor is the major mediator of the psychoactive effects of the *Cannabis sativa* plant and its derivatives. Many studies investigating cannabis abuse and psychosis have prompted debates as to whether the ECS is involved in the pathophysiology of schizophrenia (25). By acting on cannabinoid CB1 receptors, THC, the main cannabinoid found in cannabis and responsible for the majority of its psychotropic effects, interferes with brain maturation and causes long-lasting neurobiological changes when chronically administered (26, 27). THC also influences the release of neurotransmitters, such as dopamine and glutamate, that are involved in the pathophysiology of schizophrenia (28). Moreover, during adolescence, cannabis abuse has been associated with an increased risk for schizophrenia development (29). Corroborating this observation, other results also support the involvement of CB1 receptors in schizophrenia. For example, genetic associations between polymorphisms of CB1 receptors and other ECS-related genes have been related to a higher susceptibility to schizophrenia (30, 31) and response to antipsychotic drugs (32–34). Moreover, increased binding of CB1 receptor ligands has been found in the post-mortem brain of schizophrenia patients (35). It is noteworthy, however, that negative and controversial findings have also been found. For example, whereas increased levels of anandamide in the cerebrospinal fluid have been described in the prodromal stage of psychosis and antipsychotic-naïve first-episode psychosis patients (36, 37), a decrease in endocannabinoid synthesizing enzymes (NAPE and DAGL) was found in first-episode (38). These controversial data suggest that the ECS involvement in schizophrenia is complex and far from being completely understood (36, 39–41). Also, there is a lack of studies investigating changes in the ECS at different stages of the disorder.

THE CB2 RECEPTOR

The CB2 receptor shares 44% homology with the CB1 receptor (23, 42). Early studies suggested that CB2 receptors were not present in the brain but highly expressed in peripheral tissues, particularly in the immune system. Therefore, these receptors became a target for developing new pharmacological therapies to inflammatory pathological conditions, including pain, autoimmune, and neurodegenerative disorders (43–46). With the development of increasingly selective and sensitive tools, it was possible to identify CB2 receptors throughout the CNS.

CB2 receptors are expressed in the brain at lower levels than CB1 receptors, being present in glial cells, such as microglia and astrocytes, and specific subpopulations of neurons (20, 47–51). In neurons, unlike CB1, CB2 receptors are mainly expressed at postsynaptic levels, which could contribute to some of the opposite effects found after their activation (20). For example, while presynaptic CB1 receptor activation in GABAergic neurons increases the probability of postsynaptic neuronal excitation, by decreasing GABA, the activation of postsynaptic CB2 receptors usually inhibits neuronal excitability (52, 53). However, CB2 receptors located in presynaptic terminals have also been described, where, similar to CB1 receptors, they modulate neurotransmitter release (54).

Another unique feature of CB2, compared to CB1 receptors, is that they are inducible and upregulated in glial cells in response to various insults, including inflammation and chronic pain (55). In glial cells, the activation of CB2 receptors inhibits the release of several inflammatory mediators, including nitric oxide and pro-inflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6, and increases the release of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (56, 57). Also, CB2 receptors modulate the activation, proliferation, differentiation, and migration of microglia (58–60). Due to the presence of CB2 receptors in both glial cells and neurons, several groups have investigated the role of these receptors in neuroinflammation and neuroprotection (44, 56, 61, 62), and as potential targets to treat chronic neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's disease (61, 63), and psychiatric disorders, such as schizophrenia and depression (52, 64–68). A wealth of evidence indicates that inflammatory/immune changes are associated with these disorders (69, 70).

CB2 RECEPTORS AND SCHIZOPHRENIA

Accumulating evidence points that CB2 receptor-related changes are present in schizophrenia. An increase in the frequency of two single nucleotide polymorphisms (SNP) in the CB2 receptor gene (rs12744386 and rs2501432), which decrease the function of these receptors, was described in schizophrenia patients (71). More recently, a genome-wide association study of more than 120,000 participants identified an SNP intronic to the CB2 receptor gene highly associated with distressing psychotic experiences (72). In addition, non-treated first-episode psychosis and acute schizophrenia patients treated with antipsychotics showed a decreased peripheral expression of CB2 receptors than to healthy controls (38, 40). However, there has been a lack of post-mortem and neuroimaging studies evaluating the expression of CB2 receptors in patients with schizophrenia.

The preclinical studies suggesting the involvement of CB2 receptors in key neurotransmitter systems associated with schizophrenia have been recently reviewed (64). In the present paper, in addition to address these studies, we further discuss the role of CB2 receptors in inflammatory and stress-associated neuroplastic processes that have also been associated with this disorder.

CB2 Receptors in Animal Models of Schizophrenia Based on Dopamine Dysregulation

Dysregulation of the midbrain dopamine system, characterized mainly by a striatal hyperdopaminergic state, is a hallmark of the pathophysiology of schizophrenia (73). This hyperdopaminergic state is implicated in psychotic symptoms, which involve perceptual disturbances (hallucinations) and fixed beliefs resistant to contradictory evidence (delusions).

Excitatory, inhibitory, and modulatory inputs control the dopamine neurotransmission by modifying its release, postsynaptic effects, and neuronal firing patterns (74). In general, whereas glutamatergic inputs onto dopamine neurons increase excitability, GABAergic inputs inhibit dopamine neuronal function (75, 76). In addition, autoregulation of dopamine release can occur through presynaptic D2 receptors. The activation of these receptors results in inhibitory feedback that decreases dopamine release (77).

Several studies indicate that the ECS modulates the midbrain dopamine system and dopamine-related behaviors (78–80). These studies have mainly focused on CB1 receptors because, as discussed above, CB2 receptors have long been considered as peripheral cannabinoid receptors (42). CB1 receptors are expressed at low to moderate levels throughout the mesolimbic dopamine pathway. They are also highly expressed in the medial PFC (24), where they can modulate dopamine transmission (81). In the ventral tegmental area (VTA), CB1 receptors are expressed presynaptically in glutamatergic and GABAergic terminals, modulating dopamine efflux in striatal regions (82). Based on this evidence, the CB1 receptor was proposed as a promising target for treating psychiatric disorders associated with dopamine dysregulation, such as schizophrenia and drug abuse (83). However, studies with the CB1 receptor antagonist rimonabant, although yielding to promising findings on psychostimulant addiction (84), revealed that this drug induces significant adverse effects, including depression and suicide ideation (85), which limited its therapeutic use.

Similar to CB1, CB2 receptors also modulate the dopamine system. Animals lacking CB2 receptors (CB2KO) present a decrease in basal motor activity, disruption in the prepulse inhibition (PPI) test, cognitive impairments, and enhanced response to acute cocaine (66). This behavioral profile is commonly associated with symptoms of schizophrenia. Chronic treatment with the second-generation antipsychotic risperidone attenuated the PPI deficits in CB2KO mice (66). Besides, the pharmacological blockade of CB2 receptors in the nucleus accumbens (NAc) by the local infusion of the CB2 receptor antagonist AM630 increased locomotor activity and extracellular NAc dopamine levels in wild-type and CB1 receptor knockout (CB1KO), but not in CB2KO mice (79). On the other hand, similar to antipsychotics (86), drugs that activate CB2 receptors, such as the CB2 receptor agonist JWH133, attenuate cocaine-induced increased locomotor activity and its rewarding properties (87). Also, Xi et al. (79) found that JWH133, in a dose-dependent manner, inhibited cocaine self-administration, and cocaine-enhanced locomotion and NAc dopamine levels in wild-type and CB1KO, but not in CB2KO mice. In addition, JWH133 prevented the acquisition and expression of cocaine sensitization in mice. Both effects were blocked by the CB2 receptor antagonist AM630 (88). Overall, these pieces of evidence indicate that CB2 receptors modulate dopamine function and its related behaviors. However, the mechanisms by which this modulation occurs are not yet completely clear.

CB2 receptors are present on the cell body of dopamine neurons in the VTA and on the terminal of these neurons in the NAc (89–91), where they can colocalize with D2 receptors (89). Functionally, mice with a selective deletion of CB2 receptors in VTA dopamine neurons (DAT-Cnr2 cKO) present a greater locomotor response to the acute administration of amphetamine

and cocaine than wild-type animals (78). DAT-Cnr2 cKO mice also show enhanced cocaine-induced conditioned place preference and stereotypical behaviors, indicating that these receptors play a role in the VTA (92). Also, behavioral changes associated with the negative symptoms of schizophrenia were found in DAT-Cnr2 cKO mice, including anhedonia and enhanced behavioral despair (92). On the other hand, mice overexpressing CB2 receptors display an opposite behavioral profile, with lower locomotor response, self-administration, and place preference caused by cocaine (89).

In the VTA, CB2 receptors expressed in dopamine neurons can modulate dopamine neuronal excitability. Electrophysiological studies indicated that activation of CB2 receptors by JWH133 inhibits VTA dopamine neurons firing *in vivo* and *ex vivo*. Also, the infusion of this CB2 receptor agonist into the VTA and NAc inhibited cocaine self-administration and cocaine-enhanced extracellular dopamine levels. These effects were not seen in CB2KO mice and after the pretreatment with a CB2 receptor antagonist in wild-type mice (90, 93). JWH133 also decreased glutamatergic synaptic transmission in VTA dopamine neurons. However, the pharmacological blockade of synaptic transmission did not prevent the inhibitory effect of JWH133 on dopamine neuronal activity (93). Therefore, CB2 receptor activation does not impair the glutamatergic excitatory input to dopamine neurons and could directly modulate VTA excitability. Corroborating this possibility, the activation of postsynaptic CB2 receptors (a $G_{i/o}$-coupled receptor) in VTA dopamine neurons reduces intracellular cAMP levels and enhances K^+ channel function, decreasing the excitability of these neurons (93). In addition, Foster et al. have recently shown that the activation of muscarinic M4 receptors on D1 receptor-spiny projection neurons increases the release of 2-AG. Through the activation of CB2 receptors located in presynaptic terminals of dopamine neurons, this endocannabinoid causes a sustained inhibition of dopamine release. The authors have also described that the activation of M4 receptors reverses PPI disruption, an effect blocked by CB2 receptor antagonism (94). Taken together, these results indicate that CB2 receptors modulate dopaminergic transmission and, therefore, could be a promising target for the treatment of mental disorders associated with dopamine dysregulation, such as drug abuse and schizophrenia (**Figure 1**) (64, 66, 68, 80). Additional studies are needed to fully elucidate the modulatory role of CB2 receptors on dopamine function and how their pharmacological manipulation could help treat psychiatric disorders such as schizophrenia. Moreover, the impact of repeated treatment with CB2 receptor agonists on dopaminergic neurotransmission also needs to be further investigated.

CB2 Receptors in Animal Models of Schizophrenia Based on NMDA Receptor Hypofunction

Ketamine and phencyclidine (PCP) induce schizophrenia-like signs in healthy subjects (95) and exacerbate schizophrenia symptoms in schizophrenia patients (96). Since ketamine and PCP act mainly as NMDA receptor antagonists, these clinical observations led to the proposal that a hypofunction of NMDA receptors may underlie schizophrenia symptoms. Unlike drugs that enhance dopamine neurotransmission, which induce only psychotic symptoms, ketamine and PCP evoke behavioral changes associated with not only the positive but also the negative and cognitive symptoms observed in schizophrenia patients (96). In rodents, acute or repeated administration of NMDA receptor antagonists such as ketamine, PCP, and MK-801, have been used to model schizophrenia (97). The schizophrenia-like signs induced by these drugs are proposed to depend on NMDA receptors blockade in parvalbumin containing inhibitory GABAergic interneurons (98, 99). A decrease in parvalbumin expression is one of the most robust findings in post-mortem brains of schizophrenia patients (100). This decrease is also described in several animal models of schizophrenia (101), including those based on NMDA receptor hypofunction (102, 103). The functional loss of theses interneurons could result in the dopamine dysregulation and cognitive deficits seen in schizophrenia.

The acute administration of NMDA receptor antagonists induces hyperlocomotion and PPI deficits in rodents. CB2 receptor agonists were found to either attenuate or reverse these changes. For example, the CB2 receptor agonist JWH105 reversed MK-801-induced PPI deficits. Supporting the involvement of CB2 receptor, JWH105 effects were blocked by the CB2 receptor antagonist AM630, but not by the CB1 receptor antagonist AM251 (104). As expected, contrary to the effects of the CB2 receptor agonists, the blockade of CB2 receptors exacerbates both the PPI impairments and increased the locomotor activity induced by MK-801 (71, 105).

Numerous preclinical and clinical studies have indicated that cannabidiol (CBD), the major nonpsychotomimetic compound found in the Cannabis plant, presents antipsychotic properties (106). Several pharmacological targets have been suggested to mediate CBD effects (107), including CB2 receptors (108, 109). In a recent work from our group, however, a CB2 receptor antagonist failed to reverse the positive effects of CBD on the memory and social interaction deficits caused in mice by repeated treatment with MK-801 (110). In this study CBD was administered after the treatment with NMDA receptor antagonist. In a previous study we found that CBD prevents the behavioral deficits and microglial activation caused by 28 days of daily MK-801 administration (111). The involvement of CB2 receptors in this preventive effect has not yet been investigated.

CB2 Receptors as Targets for Controlling a Pro-inflammatory State in the Schizophrenic Brain

Besides the widely accepted hypotheses based on dysfunctions in dopamine and GABA-glutamate systems, dysregulation of the immune system has also been associated with the pathophysiology of schizophrenia (112).

In a healthy brain, constitutive cytokines play an important role in physiological and functional processes such as brain development, neurotransmission, and cognition (113–115). Under normal and pathological conditions in the CNS,

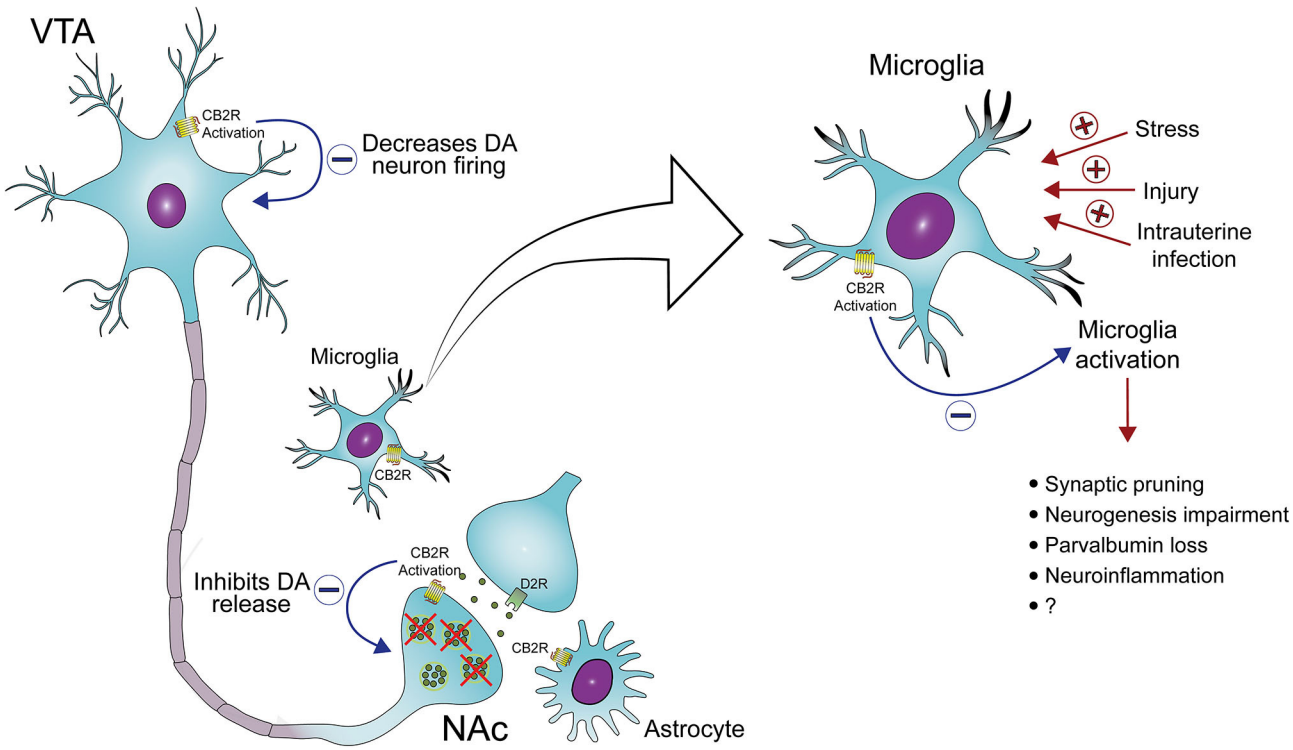

FIGURE 1 | CB2 receptors as a target to treat midbrain dopamine system dysregulation and neuroinflammation in schizophrenia. The activation of CB2 receptors located in the cell body of ventral tegmental area (VTA) dopamine (DA) neurons and the terminal of these neurons in the nucleus accumbens (NAc) decreases DA neuron firing and DA release, respectively. In addition, the activation of CB2 receptors in microglia decreases the release of pro-inflammatory mediators and, possibly, microglia-mediated neurotoxicity. Several risk factors for schizophrenia, such as stress and maternal immune activation, lead to microglia activation, which has been associated with abnormal synaptic pruning, neurogenesis impairment, deficits in parvalbumin expression, and neuroinflammation, all common findings in schizophrenia.

cytokines are produced mainly by microglia and astrocytes (116, 117). Microglia are the CNS resident macrophages and play an important role in innate immunity, rapidly responding to any pathological changes in the brain. In normal conditions, microglia contributes to synaptic development and plasticity promotes neuronal survival, and always monitors the environment by continually moving their processes (118). Prolonged microglia activation might cause brain injuries. For example, increased microglia activation during brain development may lead to abnormal synaptic pruning, which has been associated with schizophrenia (119). Besides, increased microglia activation may result in expression deficits in parvalbumin containing interneurons and in their perineuronal nets (120).

Schizophrenia patients show increased serum levels of pro-inflammatory cytokines such as IL-2, IL-6, and IL-8 (121, 122). Elevated IL-1β levels were also found in the cerebrospinal fluid of drug-naïve patients (123). Moreover, infections during the perinatal period lead to maternal immune activation characterized by a marked increase in pro-inflammatory cytokines. It may disrupt neurodevelopmental processes in the fetus and be associated with a greater risk for schizophrenia development (124–126).

Increased microglia density and in markers of microglia activation have been reported in the post-mortem brain of schizophrenia patients (127). In addition, neuroimaging studies have revealed an overactivated state of microglia in schizophrenia patients (128, 129). This state has been correlated with positive symptoms and disease duration (130). Thus, the appropriate control of microglial activation might be a promising therapeutic strategy for schizophrenia. In accordance with this proposal, some reports have demonstrated antipsychotic-like effects of minocycline, an inhibitor of microglial activation. Adjunctive therapy of minocycline to antipsychotics was beneficial in animal models and schizophrenia patients, especially against negative symptoms (131–134). Other studies, however, have failed to show any beneficial effect of this treatment (135). Furthermore celecoxib, an anti-inflammatory drug, used as an add-on medication to antipsychotics chronic schizophrenia effectively treated positive symptoms (136, 137). Taken together, these studies suggest that, even if it is still unknown whether the immune dysfunction seen in schizophrenia is a primary factor or a secondary consequence, controlling this dysfunction could be beneficial.

The expression of CB2 receptors in microglia is modified depending on their activation, being low in the healthy brain,

and high under pathological conditions (138, 139). Several studies indicate that CB2 receptor activation inhibits microglia-mediated neurotoxicity and reduces pro-inflammatory cytokine levels (140). When exposed to injury or infection, the resident microglia, similar to what occurs with macrophages, polarizes toward a pro-inflammatory phenotype (M1), characterized by the production of pro-inflammatory cytokines and antigen presentation. After activation, the M2 phenotype facilitates the resolution of the inflammatory state, through anti-inflammatory cytokines, establishing homeostasis (141). CB2 receptor activation facilitates microglia transformation from M1 to M2 phenotype, leading to a reparative scenario (142). On the other hand, CB2 receptor deletion exacerbated neuroinflammatory response in animal models of experimental autoimmune encephalomyelitis and cerebral ischemic/reperfusion injury (143–145). Thus, CB2 receptors seem to play a prominent role in inflammatory responses in the CNS. Its upregulation and activation may facilitate the downregulation and control of inflammatory processes (146). In agreement with this proposal, Ehrhart and colleagues showed that the CB2 receptor agonist JWH015 reduces IFN-γ-induced upregulation of CD40 expression in mouse microglia, which is involved in pathological activation of these cells (60).

In an animal model of Parkinson's disease, CB2 receptor activation reduced the neuroinflammatory process, brain-blood-barrier damage and T-cell infiltration, and increased nigrostriatal dopamine neuronal survival (147). *In vitro* studies demonstrated that the selective CB2 receptor agonists JWH133 and HU-308 reduced pro-inflammatory cytokines release in microglia culture (148, 149). The treatment with HU-308 decreased striatal neuroinflammation in a rodent model of L-dopa induced dyskinesia (150). This anti-inflammatory-like effect induced by the activation of CB2 receptors is also seen after a traumatic brain injury. The treatment with a selective CB2 receptor agonist decreased macrophage infiltration and pro-inflammatory cytokine expression, and increased M2 macrophage polarization (151). Other *in vivo* studies also demonstrated an anti-inflammatory effect of CB2 receptor activation in different animal models of neurodegenerative diseases (152–154).

In summary, some schizophrenia patients present marked microglia activation and increased levels of pro-inflammatory markers. The modulation of these changes as a strategy to treat this disorder seems promising (146). Given that the activation of CB2 receptors leads to the inhibition of microglial activation and the release of pro-inflammatory cytokines (65), these receptors have emerged as potential therapeutic targets (**Figure 1**).

CB2 receptors also seem to play a role in stress regulation. In mice, deletion of CB2 receptors increases stress responsivity (66) and stress exposure decreased hippocampus CB2 receptor expression (67). On the other hand, the genetically-induced overexpression of CB2 receptors produced anti-stress effect (68). In addition, the activation of CB2 receptors also induces anti-stress effects in rodents (65, 68, 155, 156). Exposure to stress, a well-known risk factor for the development of schizophrenia (157), increases microglia activation (158). Individuals at high risk of developing schizophrenia show increased responsivity to stress and are more likely to develop the disorder if they have decreased tolerance to stress (159). In animal models, stress relief during adolescence prevented the development of a schizophrenia phenotype at adulthood (160). Thus, the activation of CB2 receptors, due to its anti-stress effects (65, 68, 155, 156), may represent a strategy to prevent the transition from a high-risk state to full-scale schizophrenia. CB2 receptor may also be associated with anxiety and depression symptoms, which are clinical manifestations present in schizophrenia. A detailed discussion on this possibility was recently reviewed by Banaszkiewicz et al. (64).

CB2 Receptors, Neurogenesis, and Synaptic Plasticity

Neuroplastic changes have also been associated with schizophrenia (161, 162). For instance, impaired adult hippocampal neurogenesis, which correlates with reduced cognitive function and affective symptoms (163), has been observed in patients with this disorder (164, 165). Corroborating these findings, *in vitro* models of hippocampal neurogenesis using fibroblasts-derived induced pluripotent stem cells (iPSCs) indicated that iPSCs from schizophrenia patients showed deficits in the generation of hippocampal granule neurons with lowered levels of adult neurogenesis-related genes (166). In addition, the lack of genes thought to regulate neurogenesis produced schizophrenia-like changes in mice (167).

Some authors suggest that impaired hippocampal neurogenesis may act as a susceptibility factor for schizophrenia development, then repairing and boosting neurogenesis may be beneficial (168). Preclinical studies have indicated a neuroprotective role of CB2 receptors against impaired adult hippocampal neurogenesis (169). Activation of these receptors also enhances the proliferation of embryonic and hippocampal neural progenitor cells and may increase neurogenesis (170, 171). Thus, CB2 receptor activation might improve cognitive deficits and affective schizophrenia symptoms through neuroprotective mechanisms against impaired neurogenesis. Corroborating this possibility, we have recently found that repeated CBD prevents synaptic remodeling and the decrease in hippocampal neurogenesis caused by chronic stress (108). In the hippocampus of stressed mice, CBD enhanced the branching and number of dendrite spines and increased the proliferation and migration of newborn granule cells. These effects were prevented by co-administration of the CB2 receptor antagonist AM630 (108). Similar effects have been described after clozapine administration (172). It remains to be further investigated if these CB2 receptor-mediated effects could play a role in schizophrenia by preventing stress-induced neuroplastic changes in susceptible individuals.

CONCLUSION

Schizophrenia is a multifaceted disorder and is improbable that a single drug could adequately treat all its manifestations. So far, the available drug treatments have focused on trying to restore the hyperdopaminergic state seen in the disease. This approach is unmistakably insufficient in most patients and probably reflects the multifactorial pathophysiology of this disorder. A

complementary approach would be to act on several targets involved in complex disorders. This approach could explain why clozapine, a multi-target compound, is still the more efficacious antipsychotic drug available (173).

After thirty years of their discovery, it has become clear that endocannabinoids play a fundamental modulatory role over not only several neurotransmitter systems and cellular processes such as immune responses that can play an important role in psychiatric disorders. As discussed above, the involvement of CB1 receptors in schizophrenia is still controversial. CB2 receptors, on the other hand, seem to modulate some of the critical processes associated with this disorder, meaning the dopaminergic, glutamatergic, and immune systems (see **Figure 1**). The potential of new therapies focused on these receptors needs to be further evaluated, particularly after long term administration in models based on neurodevelopmental disruption. In addition, given its role in regulating stress and neuroinflammation, the CB2 receptors may be more critical in early psychosis development than in chronic states.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Cannabis Use in Pregnant and Breastfeeding Women: Behavioral and Neurobiological Consequences

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Nowadays, cannabis is the most consumed illicit drug. The global prevalence of the use of cannabis in 2017 was estimated in 188 million of people, 3.8% of worldwide population. Importantly, the legalization of cannabis in different countries, together with the increase in the apparent safety perception, may result in a great variety of health problems. Indeed, an important concern is the increase in cannabis use among pregnant and breastfeeding women, especially since the content of delta9-tetrahidrocannabinol (THC) is currently around 2-fold higher than it was 15–20 years ago. The purpose of this study was to review cannabis use during pregnancy and breastfeeding including epidemiological aspects, therapeutic or preventive strategies, and experimental considerations and results from animal models of perinatal cannabis exposure to analyze the underlying neurobiological mechanisms and to identify new therapeutic approaches. A recent report revealed that among pregnant women aged 15–44, last month cannabis use prevalence was over 4.9%, raising to 8.5% in the 18–25-year-old age range. Pre- and post-natal exposure to cannabis may be associated with critical alterations in the newborn infants that are prolonged throughout childhood and adolescence. Briefly, several reports revealed that perinatal cannabis exposure was associated with low birth weight, reduction in the head circumference, cognitive deficits (attention, learning, and memory), disturbances in emotional response leading to aggressiveness, high impulsivity, or affective disorders, and higher risk to develop a substance use disorder. Furthermore, important neurobiological alterations in different neuromodulatory and neurotransmission systems have been associated with cannabis consumption during pregnancy and lactation. In spite of the evidences pointing out the negative behavioral and neurobiological consequences of cannabis use in pregnant and breastfeeding women, there are still limitations to identify biomarkers that could help to establish preventive or therapeutic approaches. It is difficult to define the direct association specifically with cannabis, avoiding other confusing factors, co-occurrence of other drugs consumption (mainly nicotine and alcohol), lifestyle, or socioeconomic factors. Therefore, it is necessary to progress in the characterization of short- and long-term cannabis exposure-related disturbances.

Keywords: cannabis, tetrahydrocannabinol, pregnancy, breastfeeding, mother, offspring

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INTRODUCTION

Cannabis sativa contains more than 400 active chemicals and over 100 unique cannabinoids (1), the most prominent being trans-Δ-9-tetrahydrocannabinol (THC) as the main psychoactive constituent and cannabidiol (CBD) also produced in high concentrations but without abuse liability (2–5). The effects induced by cannabis use are mediated by the endocannabinoid system (ECS), mainly through two transmembrane domain and G-protein-coupled receptors (GPCRs), cannabinoid receptor type 1 (CB1r), and type 2 (CB2r).

Nowadays, various types of preparations of *C. sativa* are estimated to be consumed by 200–300 million people around the world, particularly among the young people (6, 7). It is the most popular illicit drug of the twenty-first century (United Nations Office on Drugs and Crime, UNODC) (8). Unfortunately, due to this growing demand in recreational activities, consumption trends increase rapidly and unexpectedly promoting the development of new synthesized cannabinoids substances (e.g., K2, spice) and certain modifications of the plant, especially those involving the increase in the concentration of THC to satisfy market expectations. For instance, values of THC were below 2% before 1990s; however, in 2017, there was a strain whose content was modified to reach concentrations between 17 and 28% (9). In addition, according to a recent study in Europe, the mean THC concentration was doubled between 2006 and 2016 both in the resin (from 8 to 17%) and in the grass (from 5 to 10%) of *C. sativa* plant (10). These changes cause greater potency in the negative psychoactive effects than those usually caused by cannabis itself (11).

The current legal landscape surrounding cannabis is surprisingly complex and unsettled. For example, 11 states and several municipalities of the United States (US) legalized medical cannabis (12). Furthermore, in Latin America, there are seven countries with a permissive legislation regarding the license for the use of cannabis (Chile, Peru, Mexico, Colombia, Bolivia, Argentina, and Uruguay, the latest being the first country in the world to legalize the cultivation and sale of cannabis in 2013) (13). The emergence of more permissive laws has led to the misperception of cannabis as a harmless substance, which is a major potential risk. A concerning study registered the incidence of cannabis use in children and teenagers aged 0–19 years from Massachusetts (98 calls were single substance and 120 polysubstance). The exposure cases were higher in male individuals (60.6%) than female individuals (39.4%) (14).

Certain reports show that nearly 10% of cannabis users consume this drug for medicinal purposes (15). In this regard, a series of randomized clinical trials have been developed with the purpose of investigating the short-term efficacy of smoked cannabis for neuropathic pain (16, 17), as an appetite stimulant especially for AIDS patients (18) or as an antiemetic drug in cancer chemotherapy (19). Notwithstanding the short-term efficacy for nausea, a recent approved and worrying application of medical cannabis is the alleviation of morning sickness and nausea in pregnant women (20, 21). Despite the difficulties to measure prenatal cannabis use (22), recent studies report that prevalence of cannabis use by pregnant women is increasing, and almost daily use was reported (16.2%) (23). Census divisions in the Midwest and West of US recently experienced the fastest changes among cannabis use treatment admissions of pregnant women (24). According to a study performed from 2018 to 2019, the consumption during the year before pregnancy increased daily from 1.17 to 3.05%, weekly from 1.39 to 2.73%, and monthly from 4.26 to 6.74%. Additionally, during pregnancy, daily use increased from 0.28 to 0.69%, weekly from 0.49 to 0.92%, and monthly from 1.18 to 1.77% (25).

THC and other cannabinoid compounds rapidly and efficiently cross the placenta and accumulate into the breast milk of nursing mothers (26, 27) producing multiple dose-dependent abnormalities in rodents (28). However, there are limited clinical reports evaluating the teratogenesis potential in exposed human fetuses or the neurodevelopmental alterations induced in lactating infants exposed to cannabis. Meanwhile, the mechanisms underlying the effects of cannabis on pregnancy and pregnancy outcome are poorly understood. It is important to mention that epigenetic modifications triggered by environmental factors during early life such as cannabis exposure might be related to the development of neuropsychiatric disorders in later life stages (29–32). Thus, clinical and preclinical studies are warranted to improve the knowledge regarding the potential negative consequences of perinatal cannabis use, particularly taking into consideration the actual legal and social cannabis landscape.

CANNABIS USE DURING PREGNANCY

Critical Involvement of the Endocannabinoid System in the Female Reproductive System and the Fetus Development

The ECS is critically involved in human fertility, and its components (enzymes, ligands, and receptors) are found in reproductive structures. Anandamide (AEA) is present in the human ovary, playing a crucial role in folliculogenesis, preovulatory follicle maturation, oocyte maturity, and ovulation (33, 34). AEA concentrations in follicular fluid appears to be correlated with oocyte quality and maturation. In this context, recent human studies indicated that plasmatic concentrations of AEA fluctuate during the menstrual cycle and the first stages of pregnancy. Clinical data suggest that high plasmatic concentrations of AEA are required for the ovulation, whereas in the period of embryo implantation and maturation, fatty acid amide hydrolase (FAAH) activity is upregulated (34). Indeed, high plasmatic AEA concentrations due to low FAAH activity in peripheral lymphocytes are predictive of spontaneous miscarriage (35, 36). Therefore, low plasmatic AEA concentrations are necessary to achieve a successful pregnancy (37). Indeed, uterine receptivity strongly depends on AEA concentrations designing the receptive area with low AEA concentrations and non-receptive area with high AEA levels (38). 2-Arachidonoylglycerol (2-AG) distribution is similar to AEA, suggesting the participation of these ligands in the early

phases of the pregnancy and in the implantation regulation. This evidence was supported in studies where embryos were exposed to high levels of AEA showing embryotoxicity, reduced trophoblast implantation, and implantation failure (39–41). Similarly, women exposed to *in vitro* fertilization program and achieving a successful implantation present low AEA concentration associated with high FAAH concentrations in their peripheral lymphocytes (42). A high FAAH activity during the first trimester and low activity in the early second trimester represent a profertility factor and predicts a successful pregnancy. This idea was sustained in recent studies where low AEA plasmatic levels were detected in healthy women in the first trimester of gestation (35) but high levels in blood and placental tissues of women presenting spontaneous miscarriage (42). Here, decreased activity and expression of FAAH in maternal lymphocytes could act as an early marker for the first trimester miscarriage. Supporting these data, very low levels of FAAH were detected in placental tissues from women with spontaneous miscarriage (43).

ECS components were detected not only at the plasmatic level but also in the human reproductive structures. High levels of FAAH were found in the human cytotrophoblast and syncytiotrophoblast, suggesting its protective role modulating AEA concentrations and preventing AEA from crossing to fetus by the placenta (44, 45). FAAH and progesterone appear to show the same fluctuations during the menstrual cycle, indicating its correlation and implication as AEA concentrations modulators (46). Consequently, AEA levels during the period may be controlled by gonadotrophins, estrogen, or its combination (37). Furthermore, ECS receptors were detected in several reproductive organs and structures in different gestational phases, and its implication in achieving a successful pregnancy has been suggested. Both CB1r and CB2r were found in the medulla and cortex of the ovary and in the corpus luteum and corpus albicans (47). In addition, it was reported that ECS regulates a normal embryo transport via oviductal CB1r (48). These findings suggest that, under physiological conditions, ECS signaling through CB1r is crucial to various female reproductive events and for the normal fetal development.

In the human fetal nervous system, EC receptors play a crucial role in hardwiring the developing brain, and its distribution is different from that in adults, suggesting that endogenous and exogenous cannabinoids may present different actions in prenatal and adult organisms. ECS dynamically controls neuronal connectivity during prenatal development in the corticostriatal–thalamic circuitry and several cortical regions involved in psychiatric disorders. For instance, CB1r expression was detected in the fetal brain at 14 weeks of gestation (49), and CB1r gene expression was significantly increased in limbic structures such as in the hippocampus CA area and basal nuclear group of the amygdaloid complex at 20 weeks of gestation (50). In addition, elevated CB1r expression is present on several white neuronal tracts of the human fetus brain disappearing at the infancy (50). In contrast, in the adult human brain, CB1r gene expression is relatively prevalent in the frontal cortex, hippocampus, basal ganglia, and cerebellum (50, 51). Thus, CB1r expression changes dynamically across the gestational period in different brain regions, suggesting its crucial role in the fetal brain maturation. CB1r signaling controls long-range neuronal connectivity, and animal studies demonstrated that prenatal THC exposure induces alterations in the structure and function of cortical circuitry (52). These effects could be correlated with the alteration of CB1r-dependent regulation of both glutamatergic and GABAergic neuron development (52). In addition, AEA could be also involved in fetal brain development. AEA concentrations in the fetus brain are low at midgestation and increases gradually during postnatal development. However, 2-AG concentrations gradually increase during embryonic phase, reaching maximum concentrations immediately after birth while these normalize during postnatal development (53).

Consequences of Cannabis Use by the Pregnant Woman on the Fetus and the Neonate

Although the pharmacokinetics of THC in adults was studied in detail, little is known during pregnancy regarding the maternal–fetal transfer of THC. Nevertheless, studies carried out in the last years indicated that after cannabis use, THC easily passes through the placenta inducing a variety of physiological effects in the fetus. THC acts as an indirect stressor to induce distress and physiological actions in later stages of life (10, 54, 55). THC molecule is highly lipophilic and is distributed rapidly to the brain and fat of the fetus after ingestion or inhalation by the pregnant mother. After maternal cannabis consumption, THC concentrations in fetal blood are approximately one-third to one-tenth of maternal concentrations. Cannabis enhances the placental barrier permeability to pharmacological and recreational substances, resulting in a potential risk factor for the fetus. The duration and magnitude of cannabis exposure and the route of administration (oral, inhalation, and different ways of smoking) are important factors involved in overall fetal toxicity (56).

Considering the distribution of ECS components in the human fetal brain, prenatal exposure to exogenous cannabinoids may modify the maturation of neurotransmitter systems and their functions through the activation of CB1r. Indeed, the binding of THC to CB1r during gestation alters the development of central dopamine and opioid neurotransmitter systems in brain areas regulating reward and motivation, increasing the vulnerability to future drug use and addiction. Postmortem studies with human fetal brains showed that prenatal THC exposure reduces dopamine D2 receptor gene expression in the basal nuclear complex of the amygdaloid system and in the nucleus accumbens. This reduction was associated with maternal cannabis consumption and was more prominent in male individuals. This fact explains, at least in part, gender differences observed in attention, learning, and memory following cannabis exposure (57). Postmortem human studies also identified that maternal cannabis use during pregnancy affects fetal expression of opioid-related genes in areas involved in emotional regulation, reward, goal-directed behavior, and motivation. Therefore, fetal exposure to cannabis might induce alterations in the limbic organization of the fetal brain, including

mu-opioid and dopamine D2 receptor in several brain areas such as the amygdala or the striatum, increasing the susceptibility for the development of neuropsychiatric disorders later in life. These genetic alterations were associated with epigenetic changes. Cannabis prenatal exposure may induce alterations in epigenetic regulation of the dopamine D2 receptor gene in the nucleus accumbens, which was associated with increased heroin seeking during adulthood. Interestingly, some studies suggest that cannabis consumption in the prenatal period may induce epigenetic changes with immunological consequences for the offspring as well as long-term transgenerational effects.

Gunn et al. (58, 59) exhaustively reviewed the effect of cannabis use on a pregnant woman, as well as on neonatal parameters such as birth weight, head circumference and length, admission to the Neonatal Intensive Care Unit (NICU), gestational age, and preterm birth. They found that women who used cannabis during pregnancy presented a higher likelihood of developing anemia; however, no significant association was found with precipitated delivery (60), manual removal of the placenta (61), maternal diabetes, or premature onset of delivery (62), among many other postpartum negative outcomes (59). Children exposed to cannabis showed a decreased birth weight and a higher likelihood of needing NICU admission, whereas the statistical models employed by authors showed no association between neonatal length, head circumference, 1 and 5 min Apgar scores, gestational age, or fetal distress, among other studied variables (59). Nevertheless, this review was not able to distinguish the independent effect of cannabis since the selected population included individuals with polysubstance use. For this reason, Conner et al. (63) attempted to address this limitation evaluating specifically the effects of maternal cannabis use on neonatal outcomes by adjusting for confounding factors such as the consumption of other drugs of abuse (e.g., alcohol or tobacco). This review analyzed the relationship of cannabis use during pregnancy with some neonatal outcomes such as birth weight, preterm delivery, admission to an NICU, stillbirth, spontaneous abortion, Apgar scores, placental abruption, and perinatal death. Authors concluded that women who smoked cannabis only were not at risk for preterm delivery, but there was an association with lower mean birth weight and lower Apgar scores in neonates. However, authors pointed out that maternal cannabis use was not an independent factor given the confounding effect mainly of tobacco, which significantly increases the risk for adverse neonatal outcomes. Similarly, Varner et al. (64) showed that tetrahydrocannabinolic acid (THCA) was found in 2.9% of women with a stillbirth while in 1.7% of the controls, but according to the authors, this result may be confounded by exposure to cigarette smoking. Finally, other studies were consistent with no significant finding association between cannabis exposure during pregnancy and several negative outcomes on the mother (gestational diabetes or hypertension/preeclampsia) or the neonate (length of infant hospital stays, stillbirth, placental abruption, fetal anomalies, gestational age) (65–67).

The effects of prenatal cannabis exposure in humans was investigated in three major prospective longitudinal clinical studies with data on the offspring beyond the early neonatal period: (i) the Ottawa Prenatal Prospective Study (OPPS) (68–71), started in 1978 with the final objective of studying the effects of cannabis used during pregnancy in white middle-class families; (ii) the Maternal Health Practices and Child Development Study (MHPCD) (72–74), started in 1982 and focused on high-risk pregnant women with low socioeconomic status, representing both white and African American women; and (iii) the Generation R study (75–81), an ongoing population-based study from the Netherlands (for more details see **Table 1**). All these three studies assessed the effects of cannabis exposure during the gestational period on the fetus with variability on behavioral data (82) (**Figure 1**).

In the neonatal population from mothers consuming cannabis during pregnancy, several physiological and behavioral alterations were observed. Researchers of the OPPS and MHPCD studies found a relationship between prenatal cannabis use and preterm births, miscarriages, pregnancy complications, low Apgar scores, and physical abnormalities in the neonates. In addition, results from the OPPS showed a decrease in the length of gestation by 0.8 weeks associated with heavy cannabis use. In contrast, MPHCD study found an increase in birth weight in neonates exposed to cannabis during the third trimester of gestation. In the Generation R study, where the fetal growth was measured by ultrasonography, an independent effect of cannabis use was found especially when cannabis use by the pregnant mother began early in pregnancy and continued throughout the entire pregnancy. Furthermore, Generation R study assessed the effect of paternal cannabis use reporting an association with fetal growth. Fetal circulation variables were also assessed in the Generation R in neonates, showing an increase in fetal pulsatility index (variability in blood velocity in a vessel). In addition, cannabis exposure during pregnancy was associated with elevated resistance index of the uterine artery, suggesting increased placental resistance. This effect could be related with reduced oxygen and nutrients accessibility, limiting a proper organogenesis that may be detrimental for the development of the fetus nervous system (82–84). Finally, a recent population-based retrospective cohort study in Ontario (Canada) was aimed to evaluate the association between self-reported prenatal cannabis use and adverse perinatal outcomes. From a cohort of 661,617 women, 9,427 (1.4%) reported cannabis use during pregnancy, and this was associated with greater frequency of preterm birth, small for gestational age, placental abruption, transfer to a NICU, and 5-min Apgar score <4 (85, 86).

Long-Term Consequences of Prenatal Cannabis Exposure During Childhood, Adolescence, and Early Adulthood

Nowadays, the scarce clinical data regarding the long-term adverse effects of cannabis use during pregnancy on the offspring mainly come from the previously mentioned OPPS and MHPCD longitudinal studies. Apart from evaluating the consequences of the prenatal exposure to cannabis on the pregnant woman, the fetus, and the neonate, these studies also analyzed behavioral and cognitive development disturbances during childhood, adolescence, and early adulthood life stages (83, 87) (**Figure 1**).

TABLE 1 | Summary of the main methodological aspects regarding the three most important longitudinal and prospective clinical studies evaluating the effect of perinatal cannabis exposure.

STUDY	POPULATION	GOALS	FOLLOW-UP	REFERENCES
Ottawa Prenatal Prospective Study (OPPS, started in 1978)	698 middle-class, low risk pregnant women Mostly Caucasian and predominantly Canadian cohort of women	Evaluate the effects of prenatal tobacco, alcohol, and marijuana exposure	The offspring was followed until the age of 18–22 years	(68–71)
Maternal Health Practices and Child Development Study (MHPCD, started in 1982)	564 high-risk predominantly single pregnant women with low socioeconomic status Caucasian (43%) and African American (57%) cohort of women	Evaluate the effects of prenatal alcohol and marijuana exposure	The offspring was followed until the age of 14 years	(72–74)
Generation R Study (Gen R, started in 2001)	9778 women living in Rotterdam (The Netherlands) Multi-ethnic cohort of women	Ongoing population-based, large-scaled study aimed to evaluate the effects of prenatal marijuana exposure on the offspring	The offspring will be followed until early childhood	(75–81)

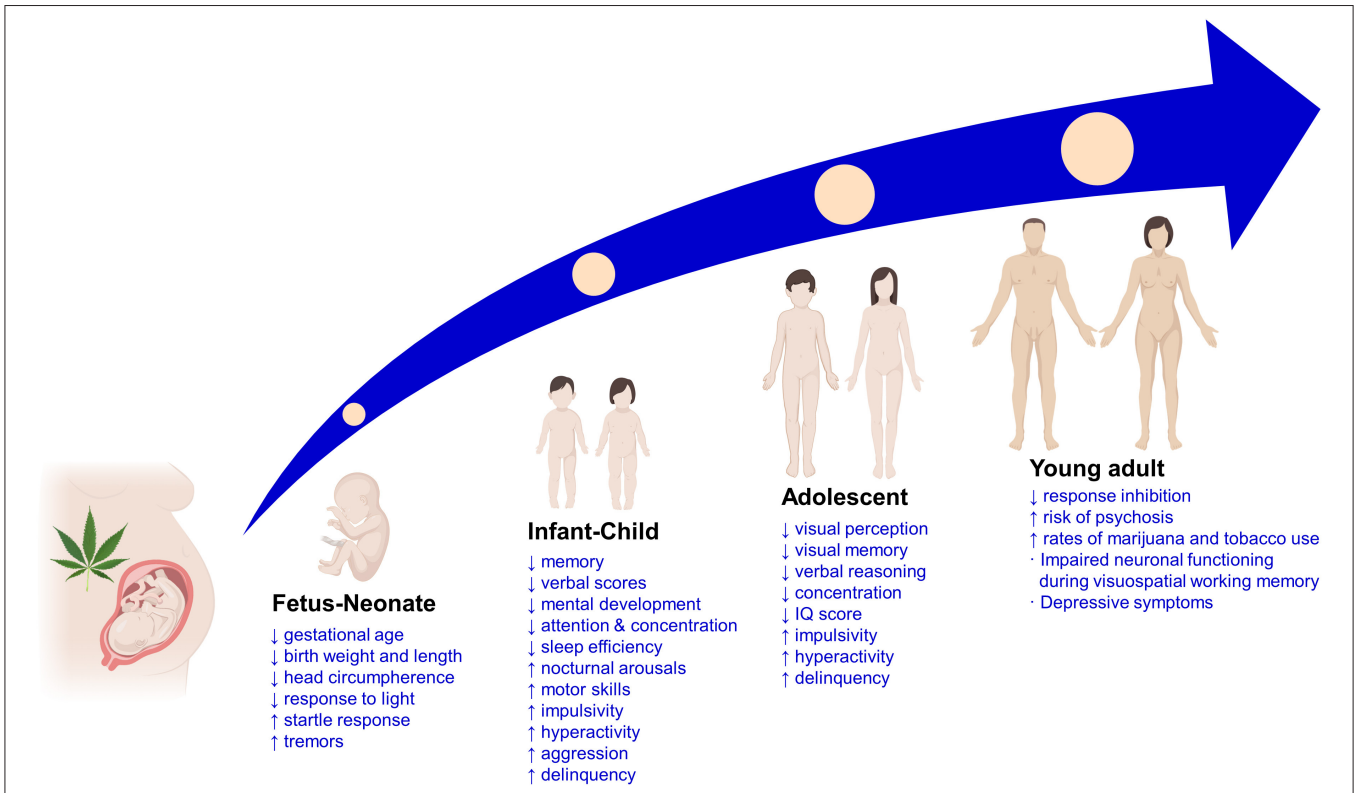

FIGURE 1 | Main clinical findings of the effects of prenatal cannabis exposure on the offspring at different life stages. ↑, increased; ↓, decreased; IQ, intelligence quotient.

Childhood

Initial observable effects in cannabis-exposed children were noticeable at 4 years of age in OPPS showing impaired mental development evaluated by means of response, memory, learning, vocalization, and verbal parameters (88). The MHPCD study detected impaired mental development at 9 months of age (89). However, these cognitive deficits were not reproduced in the Generation R study, but there was evidence of increased aggression and inattention levels in girls (79). In addition, disturbances in cognitive behavioral aspects regarding executive function domains, such as attention, planning, or working memory, were also described, entailing a significant impact on

daily life experiences. In this respect, prenatal cannabis exposure seems to critically affect attention/impulsivity and problem-solving situations that require integration and manipulation of basic visuoperceptual skills (68). Furthermore, MHPCD study provided important information regarding intellectual abilities and school achievement, revealing that cannabis exposure during the first trimester predicted deficits in reading and spelling, as well as lower child performance, whereas cannabis use during the second trimester was associated with impaired reading comprehension (90). On the other hand, both OPPS and MHPCD studies revealed that those children exposed to cannabis during pregnancy show externalizing behavior symptoms, including hyperactivity, inattention, impulsive symptoms, and delinquency (91–93). Moreover, maternal cannabis use during pregnancy was associated with the development of psychotic-like experiences in the offspring at 10 years of age (94). Despite the evidence, in a 2017 report by the US National Academies of Sciences, the committee did not identify a good- or fair-quality systematic review that reported the association between prenatal cannabis exposure and later negative outcomes for children. This could be explained, at least in part, by the critical presence of confounding factors such as the coabuse of other drugs (i.e., tobacco, alcohol).

Adolescence

Despite the high variability of results during childhood when evaluating the effects of prenatal cannabis use, there is a fair described association consistency for adolescents and young adults. Data from OPPS showed reduced visual perception and increased impulsivity at 9–12 years and decreased concentration, visual memory, and verbal reasoning at 13–16 years. Moreover, the MHPCD study revealed a decrease in abstract and visual reasoning, concentration, internalization, learning and memory, and IQ scores, along with increased externalization, depression, impulsivity, hyperactivity, and delinquency (82).

Early Adulthood

Previously mentioned deficits in executive functions associated with prenatal consumption of cannabis seem to be long lasting since 18–22-year-old young adults showed impaired neuronal functioning during visuospatial working memory processing, measured by functional magnetic resonance imaging (fMRI) (95). Furthermore, authors of the OPPS found higher rates of depressive symptoms at 16–21 years of age (96), and the MHPCD study showed increased risk of psychosis in young adults (97). Interestingly, both studies reported higher rates of cannabis and tobacco use in the exposed cohorts at ages ranging from 14–16 to 21 years (96–98).

CANNABIS USE DURING BREASTFEEDING AND ITS CONSEQUENCES

Despite the limited epidemiological data about the frequency of cannabis use during breastfeeding, a report from the state of Colorado (US) revealed that 7.4 and 4% of mothers younger or older than 30 years of age, respectively, were current marijuana users. From this population, 18% consumed marijuana during breastfeeding (99). Due to the growing trend of legalizing the recreational and medical use of marijuana, the proportion of lactating cannabis-using women worryingly increased in the last years. Furthermore, there is evidence that chronic consumption of cannabis by women, especially with a diagnosis of cannabis use disorder (CUD), does not decrease during lactation.

There is very scarce data regarding the pharmacokinetics of THC into human milk, as well as of other active cannabinoid compounds contained in cannabis. However, due to the 99% protein bound, liposolubility, and low molecular weight of THC, it could pass easily to breast milk. The first study reporting the presence of THC in mother's milk was published by Pérez-Reyes et al. (100), who detected milk THC concentrations in women actively smoking marijuana during breastfeeding up to 7.5 times THC plasma concentrations. Afterwards, other studies also evaluated the presence of THC in human milk providing interesting data regarding the elevated half-life of THC in milk and its clinical implications [for a recent review, see (101)]. In addition, it is also important to consider the infant exposure to THC by passive smoking (maternal or paternal) or by the mother's exhaled breath since THC was detected for 2 h after a single cannabis cigarette (102).

A major concern regarding cannabis use during breastfeeding is the availability of unclear, inconsistent, and even opposed information from clinical guidelines and health professionals. While some promote lactation for cannabis users independently of active use (103), others recommend the absolute cessation of cannabis use during lactation (104). Thus, there is a need to establish unified and evidence-based recommendations on the risk associated with cannabis use during breastfeeding.

There is very limited and variable evidence about the effects of cannabis use during lactation on infant development. The results from a study including 27 mothers reporting smoking marijuana during breastfeeding showed no differences in growth or mental and motor development, although infants were slightly shorter (105). On the other hand, another study with 68 infants exposed to cannabis during lactation revealed a slight and dose-dependent reduction in motor development without detecting differences in mental development in comparison with matched non-exposed infants (106). In addition, other reported effects of cannabis use on breastfed infants were sedation, growth delay, low tone, and poor sucking (107).

PREVENTIVE AND THERAPEUTIC STRATEGIES

Drug consumption during pregnancy is a major concern for mother and offspring health. Consequently, it is necessary to screen and detect the consumption of any substance of abuse among pregnant women attending prenatal units. Although its identification is still difficult, there are evidence supporting the efficacy of routine screening in clinical history or structured questionnaires in this regard (108). It is worth to mention that screening tools should be used multiple times during gestation as the patient–physician relationship progresses. Throughout

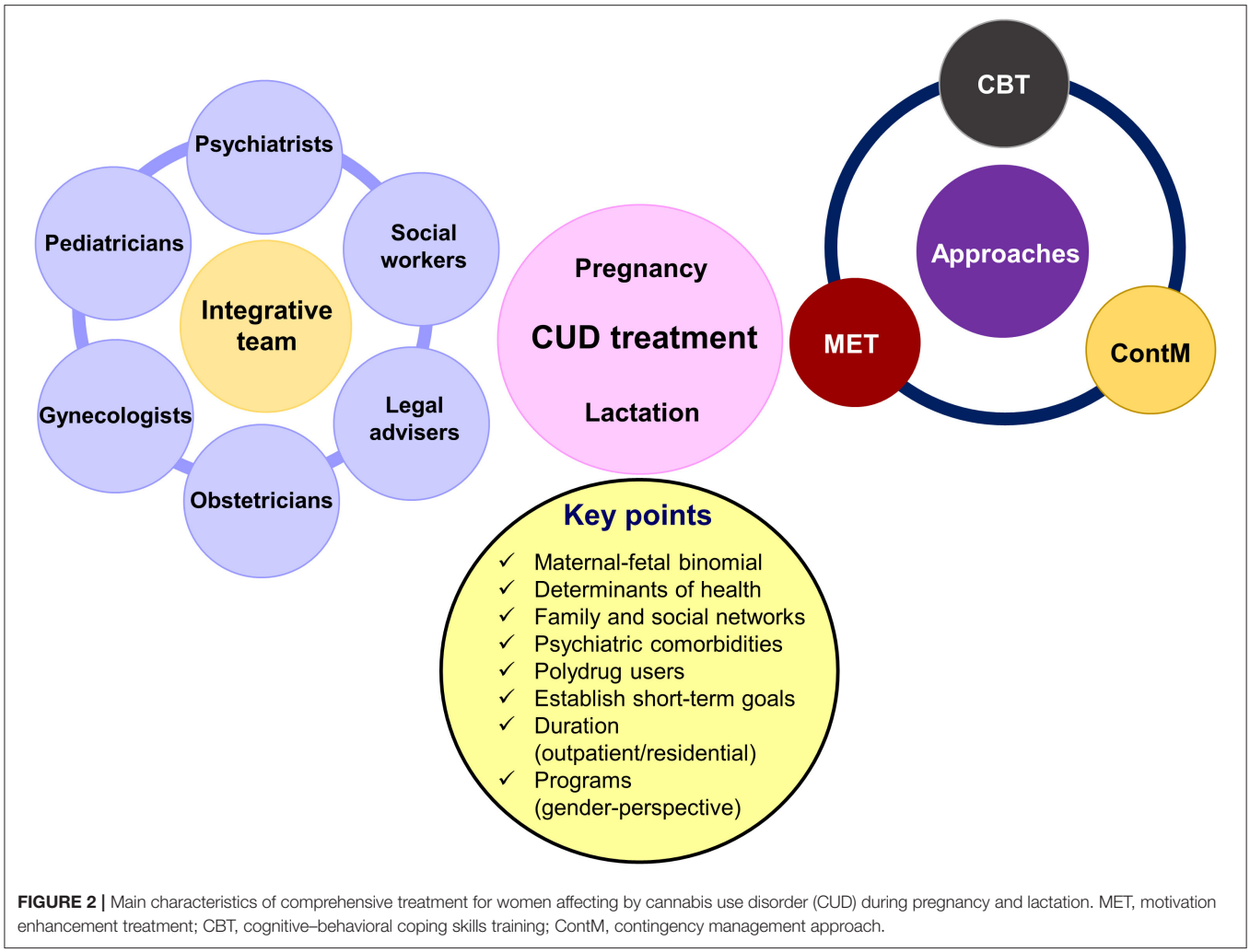

FIGURE 2 | Main characteristics of comprehensive treatment for women affecting by cannabis use disorder (CUD) during pregnancy and lactation. MET, motivation enhancement treatment; CBT, cognitive–behavioral coping skills training; ContM, contingency management approach.

the different sessions, patients are more confident with their clinician, being more open to disclose substance use problems.

In addition, toxicological screening for determining drugs and/or metabolites in maternal and neonatal biological samples is an objective and reliable approach to identify women at risk. However, in the case of cannabis, there are some limitations. Urine remains the most used sample due to its easy accessibility and the possibility of being obtained several times throughout the pregnancy. One of the main limitations of measuring THC on urine samples lies on the fact that THC can be detectable even various months after the last cannabis consumption, hampering the identification of abstinence. Meconium and umbilical cord can also be used during the second and third trimesters. However, it is not possible to identify periods of abstinence closer to delivery, apart from its limited use based on its own nature. Newborn toxicology can be used to identify families at risk of ongoing drug consumption, allowing to take actions to protect child or initiate treatment in cases of intoxication or withdrawal. If drug use disorders are not well-treated during pregnancy, the maternal difficulties handling emotions and coping with stressful situations can increase the risk of developing physiological and/or behavioral alterations in the newborn, making more difficult the postnatal adaptation of the children and the mother. Therefore, the sooner the diagnosis of cannabis abuse or dependence during pregnancy is performed, the better therapy may be planned.

CUD treatment during pregnancy is integrative including a multidisciplinary team of gynecologists, obstetricians, psychiatrics, pediatricians, social workers, and legal advisers (**Figure 2**). The most successful treatment includes combinations of motivation enhancement treatment (MET) in association with cognitive–behavioral coping skills training (CBT) and contingency management (ContM) approaches (109, 110). It is essential to adapt treatment to the needs and peculiarities of each patient. Treatment can be outpatient or residential. Long stays in care homes are a good predictor for better abstinence rates following medical discharge, less psychiatric symptoms and legal problems, and a positive attitude to child caring (111–113). Another point to highlight is the inclusion of gender perspective in the programs (114). Specific programs designed to address the special concerns of women, including the care of their children and transportation to the treatment

center, demonstrated to provide better results in comparison to traditional intensive programs. In addition, programs related to support social and familiar life improve adherence (111, 115, 116). Other strategies that provide positive outcomes are home interventions with weekly scheduled visits during the first 6 months after the delivery and then every 15 days until the year is over. Additional evidence support higher abstinence rates with home monitoring during 18 months to 3 years of duration (111, 114). The comorbidity with additional psychiatry disorders as well as polydrug use worsen patient's adherence, requiring specialized care in day hospitals or residential programs (117).

In summary, there are some key points to consider when planning treatment for women drug users during pregnancy and breastfeeding:

- Evaluate the main determinants of health such as access to health services and the socioeconomic level.
- Focus treatment on maternal–fetal binomial, bearing particularly in mind the needs of mothers to increase the motivation to achieve abstinence and not exclusively the health of the baby.
- Evaluate the family and social networks of each patient, with emphasis in the partner, to identify problems related with drug consumption and/or family violence.
- Identify comorbidities, in particular psychiatric ones.
- Avoid relapse during pregnancy and breastfeeding.
- Establish short-term goals.

Regarding breastfeeding in women with harmful use of drugs, there are for and against positions (111). The most conservative option is to discontinue lactation. Other clinicians promote continued breastfeeding except for mothers with high consumption of drugs, including cocaine, amphetamines, heroin and other opiates, benzodiazepines, or alcohol as well as in VIH+ patients. An intermediate position is to contraindicate lactation in women who consumed cannabis recently, for example in the last month previous to delivery, and to continue if patient remained abstinent during the second half of pregnancy or if she shows a clear adherence to treatment during pregnancy or postpartum. In the cases where lactation is maintained, it is advisable to make routine screening controls to stop lactation when relapse occurs.

ANIMAL MODELS OF PERINATAL CANNABIS EXPOSURE

Cannabis use among pregnant and lactating women could be recapitulated, at least in part, by preclinical experimental approaches in rodents. These models are fundamental to explore precisely and systematically the specific neurobiological mechanisms altered by cannabinoid compounds during brain development and the consequences on behavior and cognition.

Neurobiological and Behavioral Alterations

In the brain, CB1r is the main target of THC and is widely expressed through many areas of the brain during development and in the adulthood. The endocannabinoid system participates in the regulation of many brain functions including neuronal proliferation, migration, morphogenesis, and synaptogenesis, as well as in regulating the mechanisms underlying several neurological and psychiatric disorders. Consequently, it is crucial to understand the long-term effects of cannabinoid exposure at this critical stage of early brain development. Current animal studies have proved important behavioral and neurochemical alterations in several brain regions of the offspring exposed to cannabis during gestation at doses considered to be equivalent to current estimates of moderate human consumption. However, the long-lasting effects of gestational cannabinoids exposure on the adult brain of the offspring are still controversial due to the low number of studies available and the use of heterogeneous designs among studies.

Cortical neurons in the adult progeny of rat dams exposed to low doses of cannabinoids during gestation show reduced long-term depression and increased excitability. In addition, gene expression changes in metabotropic glutamatergic receptor 1/5 (mGluR1/5) and transient receptor potential cation channel subfamily V member 1 (TRPV1), as well as impaired social interaction in a sex-dependent manner (118), were also described. Furthermore, THC exposure affects cortical projection neuron development of both glutamatergic and GABAergic neurons dependent of CB1r regulation leading to impaired fine motor skills, altered corticospinal connectivity, and increased seizure susceptibility (52). In the cerebellum, maternal exposure to the CB1r agonist WIN55,212-2 affected the intrinsic membrane properties of cerebellar Purkinje neurons of the offspring and decreased the rearing frequency, total distance moved, and mobility, but a significant increase in the time of righting reflex, grooming frequency, and immobility was observed. Moreover, the neuromotor function as evaluated in the grip test and balance beam test was also affected in the WIN-treated group (119). Long-lasting alterations in GABAergic hippocampal neurotransmission was present in adult rats following perinatal cannabinoid exposure (120). In addition, reduced glutamatergic neurotransmission accompanied with a decrease in astrocyte glutamate transporters (121) and impaired cortical *N*-methyl-D-aspartate (NMDA) function has also been documented (122). These alterations may account for the altered emotional reactivity (123) and memory dysfunction observed in adult rats exposed to CB1r agonists during gestation (122).

Prenatal cannabis exposure in rodents has been associated with increased vulnerability for the reinforcing and motivational actions of certain addictive substances during adolescence and adulthood. This suggests that neurodevelopmental alterations of the endocannabinoid system may affect neurotransmitter pathways associated with reward and drug dependence. Studies using rat models of perinatal THC exposure showed an enhanced morphine self-administration accompanied with changes in mu-opioid receptor binding in female brain regions related with drug reinforcement (124). Perinatal exposure to cannabinoids altered the normal development of nigrostriatal, mesolimbic, and tuberoinfundibular dopaminergic neurons in a sex-dependent and brain region restricted manner. Cannabinoid effects were marked and constant in the striatum of male subjects while

alterations in limbic neurons were mostly transient, and those produced in hypothalamic neurons occurred after drug withdrawal (125, 126).

Most studies investigated the impact of *in utero* cannabis exposure in the offspring during the juvenile and adult age. However, the neurochemical changes that may occur during brain development at gestational ages are also essential to understand the concomitant mechanisms at this period and to determine the critical windows during gestation that are important for the long-term developmental outcome. Only few studies assessed the neurodevelopmental effects of cannabis in gestational brains. A study of Perez-Rosado and colleagues showed sex-dependent differences in the gene expression of the opioid peptide proenkephalin (PENK) in distinct regions of the fetal rat brain (127). Another study of Ana Bonnin et al. evaluated the gene and protein expression of the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH), and its activity in the brain of fetuses at different gestational days. Authors found increased TH gene and protein expression and activity at G14 compared to controls. Intriguingly, at G16, such effects were normalized, but the TH messenger RNA (mRNA) was again altered at GD18 and GD21 in a sex-dependent manner (128).

Animal studies play a pivotal role to provide critical clues regarding the neurobiological basis of perinatal cannabis exposure and its correlation with the clinical observations of the potential harmful effects of cannabis use during pregnancy. Indeed, preclinical studies suggest that the exposure to cannabinoids during pregnancy disrupts the normal brain development and produce long-lasting neurochemical changes. These phenomena may affect some behavioral traits later in life, increasing the susceptibility to develop neurological and neuropsychiatric disorders (**Figure 3**). However, the precise mechanisms require to be elucidated.

Experimental Designs

This section provides an overview of currently employed perinatal cannabis exposure rodent models, attending to the main experimental aspects, and considering its potential strengths and weaknesses (**Figure 4**).

Type of Cannabis Compound

Given the chemical complexity of the cannabis plant producing over 100 phytocannabinoids as well as the novel high herbs varieties and the new synthetic cannabinoids, it is important to consider which cannabinoid compound to select. The most widely employed phytocannabinoid is THC, the major psychoactive compound of *C. sativa* plant that binds to CB1r and CB2r. In addition, the synthetic CB1r agonist WIN55,212-2 is commonly used. Some models of perinatal cannabis exposure employ crude cannabis extract, made up of several cannabinoids, including THC, cannabidiol, and cannabinol. However, the other constituents of cannabis should be taken into consideration and administered separately to precisely uncover the harmful or beneficial effects. Nevertheless, the selection of the cannabinoid compound(s) to reproduce perinatal cannabis exposure depends on the experimental question addressed by the investigator.

Treatment

To develop an appropriate animal model, it is important to consider the differences in the developmental ontogeny. Prenatal brain development in humans does not correspond to the same developmental period in rodents. Mouse and rat postnatal period extend up to approximately 21 days, which in humans is comparable to the third trimester of pregnancy. Usually, brain maturation among species is compared using various criteria such as cerebral growth, neurogenesis, synaptogenesis, and other variables. For instance, maximal cerebral growth speed in rodents occurs up to 8–12 postnatal days while that in humans occurs in 2–3 postnatal months. Using neurogenesis as a criterion, it has been shown that E18 and E21 rat brain match with weeks 8–9 and weeks 15–16 after fertilization in the human embryo, respectively (129). In addition, differences may occur between mice and rats. Barbara Clancy et al. developed a useful online tool that translates the specific developmental time periods across mammalian species (130). Therefore, depending on the experimental question, a good translational design to study perinatal cannabis exposure from rodents to humans needs to consider these developmental differences. Furthermore, more studies are needed to simulate the type of consumer. Some users begin the consumption during the pregnancy, to diminish anxiety or nausea, and then reduce the use during the third semester or continue it during breastfeeding. Other dams are chronic users, which might cause other types of physiological and metabolic adaptations in the body that may impact differently on the fetus.

Route of Administration

The selection of a proper route of administration depends on different factors including the pharmacokinetics of the drug. Smoking is the most used route of administration by pregnant women consuming cannabis. Although this route can be simulated with inhalation chambers, animal models of perinatal cannabis exposure do not employ this design. Instead, intravenous route is the one that most closely mimics the pharmacokinetics of cannabis smoking while having the advantage of rapid response, high bioavailability, and reduced irritation in response to solutions that may contain irritant diluents. However, this route presents the difficulty of an invasive surgery and the need for trained personnel. An easier and commonly employed route is through oral, but it has certain disadvantages such as poor bioavailability, first-pass effect, and the absorption can be slower or faster depending on the stomach contents (i.e., presence of food). Subcutaneous route may also be considered. Finally, some studies used the intraperitoneal administration, but this route is not advisable for pregnant female rodents.

Period and Dose

Most of the studies perform the treatment from gestational day 5 (GD5) and prolong it to different postnatal days (PND) depending on the gestational period to be covered from a translational point of view. For instance, some studies treat animals from GD5 to PND2, which corresponds to the human midgestation (gestation week 20), although these usually extend

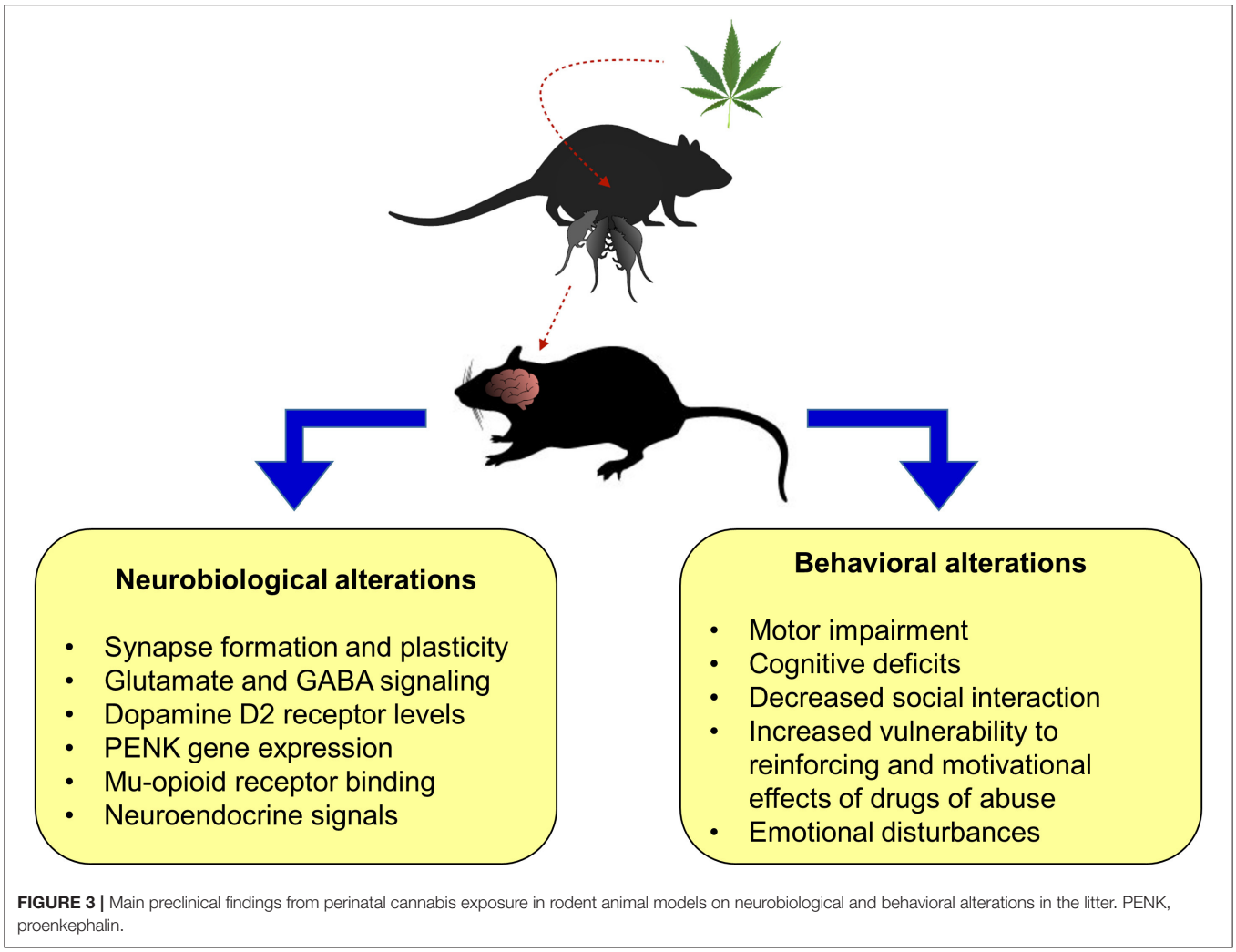

FIGURE 3 | Main preclinical findings from perinatal cannabis exposure in rodent animal models on neurobiological and behavioral alterations in the litter. PENK, proenkephalin.

cannabinoid treatment until litter weaning (PND21–24). It is not adequate to start the treatment before GD5 since there is a higher risk of spontaneous abortions.

The dosage will depend on the experimental question and the type of compound used. The doses employed in animal studies are the equivalent to current estimates to moderate human exposure, and they must be corrected by the route of administration and the body surface area. Commonly used doses for THC are 1.5–5 mg/kg (p.o. or s.c.) or 0.15 mg/kg (i.v.), and for WIN55,212-2, these are 0.5–1 mg/kg (p.o. or s.c.) or 0.15 mg/kg (i.v.). However, it is worth to mention that considering the current higher THC contents in *C. sativa* plant, animal models must be updated correspondingly.

Litter Size

The size of the litter matters especially when studying developmental mechanisms. Following birth, if the pups continue to be studied into later developmental period, a culling of litter should be performed since litter size influences pup growth and development and a number of experimental parameters (131, 132). To mention a few, body weight gain during lactation is inversely proportional to litter size, and this is associated with milk availability. Litters more than 11 pups have shown developmental delays in maturation, such as in eye opening and pinna detachment and differences in motor behavior, reflex, emotion, and memory of the offspring. Uneven growth and development can impact on the variability of statistical analysis. The number of pups born varies depending on the strain of rat or mice used. It is desirable to keep between 8 and 10 pups, and culled litters should consist of an equal number of males and females to avoid differences in maternal behavior between both sexes.

Cross-Fostering Pups

Another aspect to study is the effect of the mother on the development of the pup after birth. The cross-fostering of pups after birth avoids the confounding factor of whether the developmental alterations were potentially due to a poor maternal care or abstinence behaviors of the females exposed to cannabis during pregnancy. Therefore, a safe approach is to consider cross-fostering the litter to surrogated mothers that have not undergone to any procedure. However, when cross-fostering

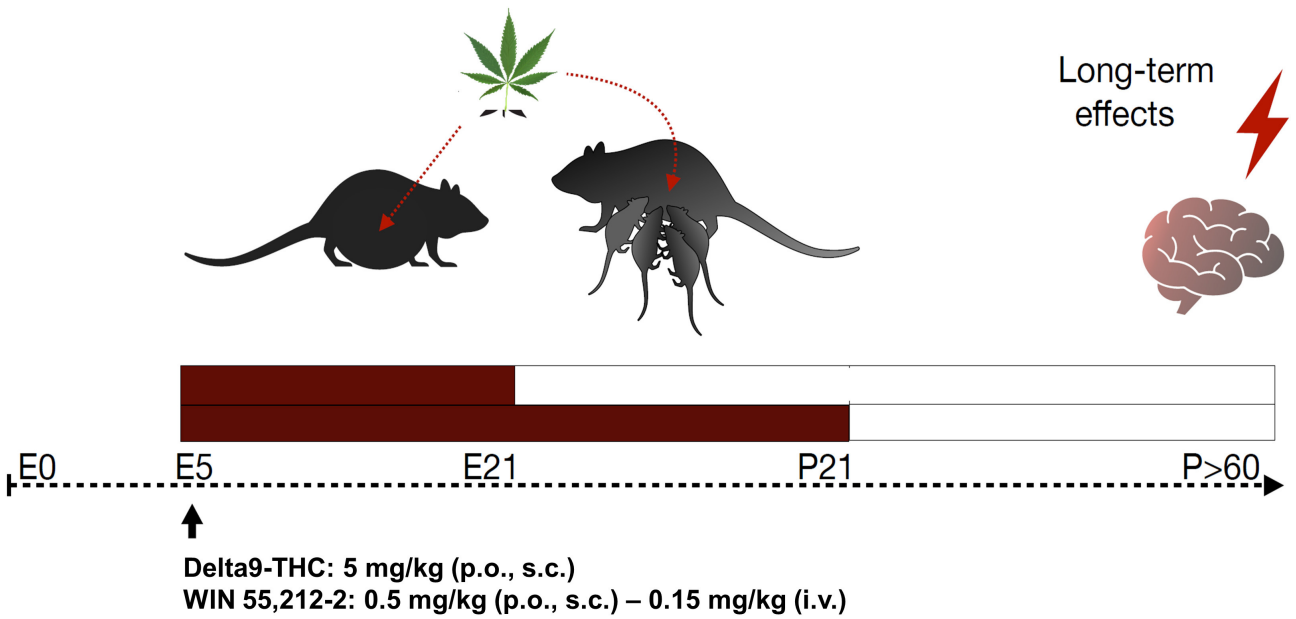

FIGURE 4 | Schematic diagram of perinatal cannabis exposure animal models and long-term behavioral and neurobiological evaluation. E, embrionary; P, postnatal; Δ9THC, delta-9-tetrahydrocannabinol; p.o., per os; s.c., subcutaneous; i.v., intravenous.

the litter, the effects of cannabis exposure during breastfeeding disappear. This depends on the experimental question since the physiological changes that may occur in the neonatal brain could be different choosing one or another experimental paradigm.

CONCLUDING REMARKS

Despite the limited information regarding the consequences of perinatal cannabis exposure on the offspring at different life stages, there is enough evidence to be aware of the potential risk of cannabis use during pregnancy and/or lactation. Considering the increasing rates of pregnant and breastfeeding women consuming cannabis due to the more permissive legislations of its recreational and medicinal uses, as well as the higher contents of THC in currently cannabis preparations, it is critical to establish preventive strategies to detect women at risk, especially with a CUD diagnosis, and to identify the most adequate interventions. Finally, the use of animal models of perinatal cannabis exposure is an essential tool to improve our knowledge regarding the underlying neurobiological mechanisms involved and to identify behavioral alterations avoiding the confounding factors present in clinical studies, mainly the consumption of other drugs of abuse.

AUTHOR CONTRIBUTIONS

FN and JM designed the sections and contents of the review manuscript. FN oversaw the organization to distribute the writing tasks among the authors and participated in manuscript writing. MSG-G, TF, AG, and AA-O perform the literature searches and participated in the manuscript writing. All authors critically reviewed and approved the final version of the manuscript.

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Prenatal Cannabinoid Exposure: Emerging Evidence of Physiological and Neuropsychiatric Abnormalities

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Clinical reports of cannabis use prevalence during pregnancy vary widely from 3% to upwards of 35% in North America; this disparity likely owing to underestimates from self-reporting in many cases. The rise in cannabis use is mirrored by increasing global legalization and the overall perceptions of safety, even during pregnancy. These trends are further compounded by a lack of evidence-based policy and guidelines for prenatal cannabis use, which has led to inconsistent messaging by healthcare providers and medically licensed cannabis dispensaries regarding prenatal cannabis use for treatment of symptoms, such as nausea. Additionally, the use of cannabis to self-medicate depression and anxiety during pregnancy is a growing medical concern. This review aims to summarize recent findings of clinical and preclinical data on neonatal outcomes, as well as long-term physiological and neurodevelopmental outcomes of prenatal cannabis exposure. Although many of the outcomes under investigation have produced mixed results, we consider these data in light of the unique challenges facing cannabis research. In particular, the limited longitudinal clinical studies available have not previously accounted for the exponential increase in (-)-Δ9– tetrahydrocannabinol (Δ9–THC; the psychoactive compound in cannabis) concentrations found in cannabis over the past two decades. Polydrug use and the long-term effects of individual cannabis constituents [Δ9–THC vs. cannabidiol (CBD)] are also understudied, along with sex-dependent outcomes. Despite these limitations, prenatal cannabis exposure has been linked to low birth weight, and emerging evidence suggests that prenatal exposure to Δ9–THC, which crosses the placenta and impacts placental development, may have wide-ranging physiological and neurodevelopmental consequences. The long-term effects of these changes require more rigorous investigation, though early reports suggest Δ9–THC increases the risk of cognitive impairment and neuropsychiatric disease, including psychosis, depression, anxiety, and sleep disorders. In light of the current trends in the perception and use of cannabis during pregnancy, we emphasize the social and medical imperative for more rigorous investigation of the long-term effects of prenatal cannabis exposure.

Keywords: cannabis, marijuana, THC, neurodevelopment, prenatal, pregnancy, placenta, cannabinoid

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INTRODUCTION

While global cannabis usage has been increasing for decades (1), more recent emphasis on the medicinal use of cannabis, and a liberalization of the political environment around cannabis, have contributed to shifts in regulatory policies. Following Uruguay, Canada became the second country to legalize the possession and sale of recreational cannabis at the federal level in October 2018 (2). Individual states in the US are also increasingly adopting more liberal recreational cannabis policies, despite illegal status at the federal level (3). It is, therefore, vital to emphasize the need for accelerated research in promoting an evidence-based approach to the rapidly changing policies and regulations regarding cannabis, particularly for sensitive subgroups, such as pregnant women.

Considerable evidence suggests that there is a fundamental lack of understanding among the general population regarding the potential risks of cannabis use during pregnancy. For example, in a recent anonymous survey from Hamilton, Ontario, an understanding that cannabis-derived phytochemicals, such as (-)-Δ9– tetrahydrocannabinol (Δ9–THC), can be transmitted to the fetus during pregnancy was insufficient in influencing the choice of whether to discontinue cannabis use while pregnant (4). These data are consistent with reports showing that, in the past two decades, the perception that cannabis use poses no risk during pregnancy has increased 3-fold among reproductive-aged women in both clinical settings and across large-scale nationally representative surveys in the US (5, 6). In particular, women who reside in areas where recreational cannabis is legalized and those who report regular cannabis use prior to pregnancy perceive far less risk of continued use during pregnancy, possibly owing to a positive perception of therapeutic effects and a lack of communication with health care providers regarding the risks (5, 7, 8). Indeed, in an online survey approximately half of the health care provider participants did not explicitly discourage prenatal cannabis use (9). This lack of perceived risk is reflected in the increasing rates of prenatal cannabis use. In North America, survey and toxicology data derived from large health care databases indicate that prenatal cannabis use increased by 62% from 2002 to 2014 (10), and by 170% from 2009 to 2016 (11). Prevalence of prenatal cannabis use also appears to be age-dependent: as low as 3% in women older than 34 years and as high as 22% in women aged 18–24 years (11), though self-reported prenatal cannabis use was as high as 35% in one relatively small sample (12). Importantly, data derived from self-reporting likely underestimates the prevalence of prenatal cannabis use due to social desirability bias, with at least one report illustrating a large disparity between self-reporting (2.6%) when compared to umbilical cord blood samples (22.4%) (13).

Several factors are related to the decision to consume cannabis during pregnancy. Self-reporting data often highlight the management of mood disorders, such as depression and anxiety, as primary reasons of prenatal cannabis use. This is consistent with data showing greater odds of cannabis use for pregnant women diagnosed with depressive and anxiety disorders (14), as well as those reporting stressful life events in the year prior to pregnancy (15). The management of nausea is another frequently reported reason for prenatal cannabis use (16). In one study, 83% of medically licensed cannabis dispensaries in Colorado recommended cannabis products to alleviate morning sickness, with the majority of recommendations based on personal opinion (17). Therefore, unlike the use of other illicit substances during pregnancy, there is a strong perceived medicinal incentive for the use of cannabis coupled with a lack of perceived risk, even among medically licensed dispensaries and health care providers. In the absence of rigorous scientific evidence and consensus on the effects of prenatal cannabis use, the aforementioned trends are, thus, likely to continue. In this review, we summarize recent clinical and preclinical data on the effect of prenatal cannabis use. In doing so, we consider neonatal outcomes, physiological effects, and neurodevelopmental outcomes. We also consider the strength of the available evidence and highlight areas of relative consensus and knowledge gaps. Summaries of the clinical and preclinical studies discussed in this review have been organized in **Supplementary Table 1**.

PHYSIOLOGICAL OUTCOMES OF PRENATAL CANNABINOID EXPOSURE

Neonatal Outcomes

Meta-analyses and reviews of the literature have previously highlighted inconsistencies in the effects of prenatal cannabis exposure on neonatal outcomes including low birth weight (LBW), preterm delivery (PTD), and neonatal intensive care unit (NICU) admission (18–20). Notably, these analyses largely focused on studies dating from the late 1980's to the 2000's. Few of these studies provided information on gestational age of exposure and frequency of use, and none accounted for dose or concentration of Δ9–THC. This is particularly relevant given that the mean Δ9–THC concentration in cannabis has doubled over the past decade (21). Large cohort studies do suggest an association between *in utero* cannabis exposure and fetal growth restriction (FGR), including decreased head circumference (22). Additionally, early studies often did not delineate the effects of prenatal cannabis use from the impact of polydrug use. Several systematic reviews and meta-analyses have indicated that cannabis use leads to FGR and postnatal neurodevelopmental outcomes, however they are confounded by sociodemographic factors and the fact that users often used other drugs (e.g., tobacco) (18, 19, 23, 24). Indeed, more contemporary studies, some of which account for multiple factors, including *in utero* exposure to other drugs (tobacco, alcohol, benzodiazepine, and opioids), race, age, medical insurance, parity, and marital status, report that prenatal cannabis exposure alone is sufficiently predictive of LBW, PTD, and NICU admission (25–29).

In animal studies, Δ9–THC doses of approximately 3 mg/kg intraperitoneal (i.p.) (both acutely and chronically administered for 21 days) result in circulating concentrations of 8.6–12.4 ng/ml Δ9–THC after a 24-h washout period, which is consistent with that reported in cannabis smokers (13–63 ng/ml from a 7% Δ9–THC content cigarette) 0–22 h post inhalation, as well as in aborted fetal tissues (4–287 ng/ml) of pregnant cannabis smokers (30–32). In preclinical studies, which are better suited to control for environmental factors such as dosing and polydrug use,

prenatal exposure to similar clinically relevant doses of Δ9–THC often recapitulate the LBW effect often reported in clinical studies (33–36). However, this effect is not always observed, with some studies reporting no effect on birth weight (37–42). This discrepancy may be related to route of administration, with LBW more often reported in studies that use i.p. injections, a lack of effect on birth weight more often reported in studies that use oral administration, and mixed results in studies that use vapor inhalation. In addition to the effects of prenatal Δ9–THC, studies are warranted to examine the safety of gestational cannabidiol (CBD; the major non-psychoactive constituent of cannabis) short- and long-term. Recent reports suggest that 62% of CBD users report pain, anxiety, and depression, all common ailments in pregnancy as reasons for use (43). In meconium and umbilical cord samples, both established markers of *in utero* cannabinoid exposure, the range of CBD is reported to vary from 10 to 335 ng/ml (44). Although there is a widespread perception that CBD is a "cure-all" to reduce these symptoms, its safety in pregnancy is unknown. Preclinical rodent studies are necessary to address the long-term effects of CBD on pregnancy and postnatal health.

Despite the higher quality of data in contemporary clinical studies, the independent and combined effects of Δ9–THC and CBD have not been delineated. This is a critical consideration given that the Δ9–THC:CBD ratios and concentrations can vary dramatically in available recreational cannabis products. In addition, CBD has been shown in clinical and preclinical studies to block or strongly mitigate the neuropsychiatric side-effects of Δ9–THC (45–47), meaning that high Δ9–THC/low CBD cannabis products may pose additional risks during prenatal development. Thus, while the preponderance of recent evidence suggests that prenatal cannabis use adversely impacts neonatal outcomes, a scientific consensus requires careful consideration of relevant variables such as polydrug use, the frequency and timing of prenatal cannabis use, and the relative chemical composition of the cannabis being consumed. Furthermore, preliminary correlational analyses highlight congenital outcomes, including cardiovascular defects, Down syndrome, and gastroschisis, which may be of importance for future investigation (48).

Placental Abnormalities

CB_1R and fatty acid amide hydrolase (FAAH), which hydrolyzes the endocannabinoid anandamide, are present in all layers of the human placenta (49). In rodent models, the ECS is present in midgestational placentas, where is has been demonstrated to play a critical role in placentation, trophoblast differentiation, as well as fetal outcomes, such as resorption rates (50). These findings highlight the importance of investigating the impact of exogenous cannabinoid exposure on placental development. The limited clinical data available demonstrate associations between prenatal cannabis exposure and increased placental weight (51), as well as enlarged umbilical vessel diameter (52). Closer examination in cultured human cells reveals that Δ9–THC hampers trophoblast remodeling through an antioxidant effect that prevents cell death of syncytiotrophoblasts (53). This is consistent with histological results from human placentas showing increased syncytiotrophoblastic knots and fibrin exudation in the villous stroma of cannabis users (34).

In rodents, prenatal Δ9–THC induces FGR with concurrent increases in placental weight and fetal to placental weight ratio (33, 36). Additionally, clinically relevant doses of Δ9–THC (3–5 mg/kg/day) lead to adverse morphological changes in placentas (34, 36). Specifically, Δ9–THC exposed animals exhibit an increase in labyrinth area (36), with increased diameters of trophoblastic septa (34). In pregnant mice given 5 mg/kg daily Δ9–THC, disordered structure of spongiotrophoblasts and decreased number of glycogen cells in junctional zone was also observed (34), although this effect was not recapitulated in rats exposed to 3 mg/kg daily Δ9–THC (36). Consistent with clinical findings of enlarged umbilical vessel diameter, maternal blood sinusoids within the labyrinth layer of exposed rats was found to be enlarged, while fetal blood space was reduced (36). Furthermore, labyrinth trophoblasts of exposed rats exhibited reduced glucose transporter 1 (GLUT1) and glucocorticoid receptor (GR) expression (36). Along with the abovementioned placental alterations, these findings implicate impaired maternal-to-fetal glucose transport as a possible mechanism of Δ9–THC induced nutrient insufficiency and FGR.

Metabolic Outcomes

The mammalian endocannabinoid system (ECS) plays crucial regulatory roles in fetal peripheral organ development (54, 55). While the exchange of endogenous endocannabinoids between the mother and fetus is tightly regulated, approximately one-third of exogenous plasma Δ9–THC from the mother crosses the placental barrier to the fetus (56). The dysregulatory impact of sustained maternal administration of Δ9–THC on fetal metabolic processes is in the early stages of investigation. In rats, prenatal exposure to Δ9–THC [partial agonist of cannabinoid type 1 receptor (CB_1R)] leads to decreased BW, brain to BW ratio, liver to BW ratio, and pancreatic weight at birth (35, 36). By 3 weeks of age, these offspring undergo postnatal catch-up growth resulting in glucose intolerance, paralleled by decreased pancreatic total and small islet density at postnatal day (PND) 21 and 5 months, specifically in female offspring (35). This is consistent with data demonstrating that endogenous regulation of CB_1R is critically involved in fetal pancreatic islet organization (55). Moreover, activation of CB_1R reduces pancreatic β-cell proliferation and impedes insulin receptor activity, while CB_1R antagonism can improve insulin resistance (57, 58). Importantly, Δ9–THC exposed rats also exhibited reduced body weight and pancreatic weight at birth, suggesting that the commonly observed clinical outcome of LBW may be associated with fetal glucometabolic dysregulation, an outcome that may disproportionately impact the long-term metabolic health of female offspring (35). While the sexual dimorphism could be attributed to differences in circulating sex hormones, the concentrations of estrogen and testosterone were not different in Δ9-THC offspring, suggesting a potential epigenetic mechanism (35). Given the links between FGR and long-term metabolic disease (59), further studies are warranted to assess if any cardiometabolic defects manifest long-term.

At the cellular level, involvement of the ECS has been demonstrated in metabolic processes relevant to fetal development. Indeed, mitochondrial and endoplasmic reticulum (ER) stress contribute to gestational complications, such as FGR (60), and Δ9–THC has been shown to decreases oxygen consumption and membrane potential of rat heart mitochondria, an effect that appears to be independent of cannabinoid receptor activation (61). Similarly, in the brain, Δ9–THC impedes mitochondrial respiratory rate, both through CB_1R and non-receptor-mediated mechanisms (62). In astroglial mitochondria, activation of CB1R hampers glucose metabolism and brain lactate production, leading to altered neuronal function and behavioral deficits (63). Recently, these effects were recapitulated in human placental BeWO trophoblast cells, where it was demonstrated that Δ9–THC treatment decreases mitochondrial respiration, as well as dose-dependently increases ER stress (64). These effects were blocked by CB_1R/CB_2R antagonism and underscore the importance of ECS homeostasis in the development of fetal energy homeostasis. Given that LBW Δ9-THC-exposed offspring exhibit postnatal catch-up growth, a driver of ER stress and mitochondrial dysfunction (65, 66), it remains possible that cannabinoids *in utero* could also *indirectly* influence the development and function of metabolic organs in postnatal life.

NEURODEVELOPMENTAL OUTCOMES OF PRENATAL CANNABINOID EXPOSURE

Cognitive Outcomes

Growing epidemiological and experimental evidence over the past two decades has demonstrated an association between cannabis use during adolescence (a critical period of neurodevelopment) and increased risk of cognitive deficits and neuropsychiatric disease (67–69). The ECS is also critically involved in fetal neurodevelopmental processes, including synaptic plasticity, as well as neuronal cell proliferation and differentiation (70, 71). Considering that Δ9–THC readily crosses the placental barrier from the mother to the fetus, these processes and their long-term cognitive outcomes are potentially vulnerable to disruption by *in utero* cannabis exposure.

To date, three large prospective longitudinal cohorts have been used to investigate the consequences of prenatal cannabis exposure on neurodevelopment: The Ottawa Prenatal Prospective Study (OPPS) (72–76), The Maternal Health Practices and Child Development Study (MHPCD) (77, 78), and The Generation R Study (GenR) (79, 80). These data highlight several cognitive and behavioral domains affected by *in utero* exposure to cannabis. Across childhood and adolescence, cannabis exposure was associated with deficits in memory, verbal reasoning, concentration, attention, and Bayley Scale of Infant Development (BSID) scores, as well as increases in hyperactivity, impulsivity, and aggression (81–83). At 10 years of age, exposure in the MHPCD cohort was also predictive of poorer academic achievement as measured by Wide Range Achievement Test—Revised (WRAT—R) reading and spelling scores (78). Additionally, functional magnetic resonance imaging (fMRI) on exposed subjects from the OPPS cohort showed altered executive function and visuospatial working memory processing into young adulthood (75, 76). However, most performance effects from these cohorts were relatively subtle, and inconsistencies were present. Indeed, a recent systematic review of these data and other smaller cohorts determined that outcomes differed on only 4.3% of cognitive measures (with cannabis exposure being associated with worse outcomes in 3.4% of cognitive measures) (84). This review also concluded that the statistical differences were not clinically significant. Importantly, however, these data are largely derived from the 3 large prospective studies, which were initiated between 1978 and 2001. Therefore, recent trends toward cannabis legalization, accompanied by increased frequency of use, and the sharp spike in Δ9–THC concentrations observed over the past two decades are largely unaccounted for in these analyses. Indeed, in a more recent retrospective observational cohort, a positive maternal Δ9–THC urine test at the first prenatal visit was associated with abnormal 12-month developmental scores in infants, as measured by the Ages and Stages: Social–Emotional Questionnaire (ASQ-SE) (27). Moreover, recent cross-sectional results from the ongoing Adolescent Brain Cognitive Development (ABCD) study, which recruited 11,875 children aged 9–11 years, found that prenatal exposure to cannabis was associated with deficits in attention, thought, and social problems after accounting for potentially confounding covariates (29). A moderate increase in the incidence of intellectual disability and learning disorders was also observed in a large retrospective analysis of children born between 2007 and 2012 in Ontario, Canada, though these results were not statistically robust (85). The cognitive impairments observed in longitudinal cohorts, though often subtle, are also corroborated by mechanistic plausibility.

For example, in a recent study, human induced pluripotent stem cells (hiPSC) induced toward neuronal commitment, thus mimicking developing fetal neurons, were exposed to Δ9–THC and CBD for 37 days (86). At the clinically-relevant doses studied, CBD produced neurotoxic effects, while Δ9–THC promoted precocious neuronal and glial differentiation, and induced abnormal functioning of voltage-gated calcium channels. Furthermore, *in utero* exposure to cannabis has been demonstrated to disrupt fetal cortical and hippocampal connectivity by activating CB_1R-mediated degradation of proteins that stabilize microtubules, effectively limiting the computational power of circuits relevant to cognitive function (87). A specific loss of cholecystokinin (CCK) interneurons in the hippocampus has also been observed in mice prenatally exposed to Δ9–THC (88). Interestingly, when systematically compared, these effects were similar to those observed in animals perinatally exposed to alcohol (89). Changes in cognitive performance have also been observed in animal models of prenatal cannabis exposure. Adolescent and adult rodents prenatally exposed to either Δ9–THC or a synthetic CB_1R agonist have been shown to exhibit impairments in learning, long-term memory, short-term olfactory memory, spatial working memory, and attention when compared to non-exposed rodents (37, 40, 90–92). Although most of this data was derived exclusively from male rats, one study that assessed both

male and female offspring found a more pronounced cognitive deficit in males (40). Importantly, these cognitive deficits were associated with cortical changes including decreased glutamate and norepinephrine, increased kynurenine, and altered neuron morphology (37, 91–94). Cognitive deficits were also associated with decreased hippocampal glutamate and γ-aminobutyric acid (GABA) outflow and uptake, decreased CB_1R expression, and impaired hippocampal long-term potentiation (LTP), a neurophysiological model for learning and memory (41, 90, 95).

Neuropsychiatric Morbidity

Cognitive deficits are often symptomatic of neuropsychiatric morbidity, and the associated brain regions and neurophysiological pathways are often implicated in disease states including schizophrenia, depression, and anxiety (96). However, to date, there has been a paucity of longitudinal data specifically assessing the effect of *in utero* exposure to cannabis on the risk of developing neuropsychiatric disease. Of the large longitudinal studies, depression was only assessed in the MHPCD cohort, where it was found that exposure was associated with a higher rate of depression in adolescence (83). In the OPPS cohort, fMRI showed a correlation between *in utero* cannabis exposure and increased neuronal activity in bilateral prefrontal cortex (PFC) during response inhibition (97). This is of note since it has been demonstrated that neural processes involved in response inhibition are abnormal in schizophrenia (98). Indeed, recent data from the ABCD study was used to determine whether prenatal cannabis exposure was associated with psychosis proneness, as assessed by the Prodromal Questionnaire–Brief Child Version total score and psychotic-like experiences (PLEs) (29, 99). These analyses found that exposed children, ages 9–11, exhibited increased vulnerability to psychosis symptoms. Consistent with these findings, an analysis of children from the GenR cohort found that *in utero* cannabis exposure was associated with child psychotic-like experiences, assessed through the Youth Self Report questionnaire (100). In adolescence, *in utero* cannabis exposure was also linked to externalizing problems (aggressive/rule-breaking behavior) in three of the four large cohorts discussed: the MHPCD, the GenR, and the ABCD cohorts (29, 77, 101). Internalization problems (anxiety/depression features, such as withdrawal) were also observed in the MHPCD and ABCD cohorts (29, 77), whereas in the GenR cohort, internalization problems were associated with smoking cannabis prior to, but not during, pregnancy (101). Interestingly, these study found a similar associations with reports of paternal cannabis use during the pregnancy, which was interpreted by the authors as suggestive of a common etiology underlying both parental cannabis use and offspring psychotic-like experiences, in contrast with a direct *in utero* causal link between exposure and offspring phenotype (100, 101). Moreover, a recent study of live births in Ontario, Canada between 2007 and 2012, reported that prenatal cannabis use was associated with increased incidence of autism spectrum disorder in the offspring, though this analysis relied on self-reported retrospective data that may have suffered from underreporting of cannabis use and other residual confounding bias (85).

Molecular data from cannabis-exposed human fetal specimens have demonstrated a dose-dependent reduction of dopamine (DA) receptor subtype D_2 in the amygdala basal nucleus, particularly in males, suggesting impairment in the mesocorticolimbic neural systems that regulate emotional behavior (102). Since tobacco co-use often occurs with cannabis use during pregnancy, it is also relevant to consider the combined effects of prenatal exposure to both of these substances. To this end, two studies have demonstrated that co-exposed infants and kindergarten aged children exhibit an attenuated cortisol response to stressors, with a greater effect observed in males (103, 104).

In support of the available clinical data, animal studies have further corroborated the association between *in utero* cannabis exposure and neuropsychiatric deficits. Early studies showed that prenatal exposure to Δ9–THC was associated with sex-specific alterations in the hypothalamus-pituitary-adrenal (HPA) axis (105). More recent data showed that PND12 rat pups prenatally exposed to Δ9–THC exhibited an increase in the frequency of ultrasonic vocalizations (USV) when removed from the nest, a behavior that is possibly analogous to human infant crying and that may indicate long-term neuro-behavioral alterations (39). When tested during adolescence and adulthood, these rats exhibited a decrease in play behavior and social interaction and an increase in anxiety-like behavior on the elevate plus-maze test (EPM), respectively. Consistent with these results, exposed adult males exhibited anxiety-like behaviors in another paradigm, the open field test (OFT), suggesting long-lasting behavioral effects of *in utero* exposure (38). The mesolimbic reward/motivation pathway may also be impacted by *in utero* exposure to Δ9–THC. Adult male and female rats prenatally exposed to Δ9–THC exhibited a dampened locomotor response to a challenge of amphetamine (40). DAergic neurons in mesolimbic pathway are involved in locomotor response to psychostimulants, such as amphetamines, suggesting a strong relevance of these behavioral outcomes to the risk of developing substance use disorders, which warrants further investigation. Indeed, early data demonstrated that exposed rats show increased self-administration of morphine, paralleled by an increase of μ opioid receptor density in the PFC, the hippocampus CA3 area, the amygdala posteromedial cortical nucleus, the ventral tegmental area (VTA), and the periaqueductal gray matter (106). Although in this study alterations were observed in exposed female, but not male, offspring, others have demonstrated an increase in the rewarding effect of morphine in exposed offspring of both sexes, with a stronger effect in males (105, 107). Many of these observed neuropsychiatric deficits may also be linked to the neurophysiological alterations discussed earlier in the context of cognitive deficits, as the brain regions (PFC and hippocampus) and neurotransmitter systems involved are commonly implicated in neuropsychiatric illness as well.

Sleep Disturbances

Studies into the role of the ECS in sleep, as well as the potential for cannabis to alleviate symptoms of sleep disorders have suggested a role for the ECS in circadian regulation (108, 109). In the MHPCD cohort, neonatal electroencephalogram (EEG)

analysis found that prenatal exposure to cannabis was associated with increased motility and disruptions in sleep and arousal (110). These disturbances persisted at 3 years of age, with exposed children exhibiting increased nocturnal arousal, more awake time after sleep onset, and lower sleep efficiency (111). However, these studies included a relatively small sample size, with 55 newborns initially assessed and 48 children assessed at 3 years of age, including non-exposed controls. More recently, child sleep outcomes were assessed in the ABCD cohort using 11,875 exposed children ages 9–10 (112). This study also controlled for multiple covariates, including mother's education, household income, parental marital status, race, and child sex. The investigators found that maternal report of cannabis use was significantly associated with symptoms of disorders of initiating and maintaining sleep, disorders of arousal, sleep wake disorders, disorders of excessive somnolence, and a summed sleep disorder score as measured by the Sleep Disturbance Scale for Children (SDSC) (112). Furthermore, children of mothers who reported daily use of cannabis during pregnancy were at increased risk of symptoms of disorders of excessive somnolence. These findings are highly suggestive of a long-lasting impact of *in utero* exposure to cannabis on circadian regulation, though further cross-study replication of these findings is needed. Additionally, there is a paucity of animal studies to address these effects and their possible neurophysiological mechanisms. Therefore, a causal link remains elusive and requires more controlled investigation.

PATERNAL CANNABINOID USE AND EPIGENETIC CONSIDERATIONS

To date, the large majority of studies examining long-term effects of exposomes in pregnancy have focused mainly on the maternal environment. As previously discussed, analysis from the GenR cohort revealed that paternal cannabis use was predictive of psychotic-like experiences and behavioral deficits in offspring at ages 7–10, independent of maternal cannabis use (100). Notably for this cohort, paternal cannabis use was derived from maternal reports, and was only determined for the pregnancy period, not prior to pregnancy. While the authors of this study hypothesized a potential common etiology underlying both parental cannabis use and offspring behavioral outcomes, it is also possible that paternal preconception use is causally associated with the observed offspring phenotypes. Recently, it was demonstrated in rats that Δ9–THC exposure during adolescence, prior to mating, may influence neurodevelopmental and behavioral outcomes in subsequent generations (113, 114). Offspring of Δ9–THC exposed parents, who were themselves unexposed, exhibited enhanced heroin self-administration paralleled by molecular and electrophysiological alterations in the striatum, a key component of the reward circuitry (113). Moreover, sex-specific effects were observed at the levels of gene expression and behavior (114). In terms of paternal cannabis use specifically, direct evidence now exists, in both humans and rats, demonstrating that Δ9–THC exposure alters DNA methylation in sperm cells (115). These alterations may represent a vector by which paternal toxicant exposure is able to influence genetic expression, and therefore development, in the offspring. Indeed, adult male offspring of premating Δ9–THC exposed fathers were shown to exhibit deficits in attentional performance and memory tasks, paralleled by alterations in acetylcholine signaling (116–118). Interestingly, both prenatal and adolescent exposure to THC has been shown to potently sensitize the brain's DA pathways, effects which persist into later life (119–121). Such Δ9–THC-induced dysregulation of mesocortical and mesolimbic DAergic transmission patterns may be critical biomarkers for not only increased addiction risks, but also an underlying mechanism linked to increased vulnerability to schizophrenia, mood and anxiety disorders. Notably, the evidence for the influence of paternal cannabis use on offspring outcomes is in the early stages and predominantly preclinical. While these studies provide an important case for biological plausibility and warrant further mechanistic exploration, clinical validation is vitally needed to parse the contributions of paternal and maternal cannabis use on offspring outcomes. Ideally, prospective investigations should, therefore, delineate offspring outcomes for paternal-only exposure, maternal-only exposure, and combined exposure from both parents.

CONCLUSION

In this review, we presented a summary of the available data on the effects of prenatal cannabinoid exposure. With an emphasis on contemporary and emerging data, we considered the impact of prenatal cannabinoid exposure on neonatal outcomes, persistent metabolic and physiological disturbances, as well as neurodevelopmental and neuropsychiatric liability. We have also considered the emerging role of paternal and cross-generational effects of cannabinoid exposure. In human studies, the preponderance of evidence suggests that prenatal cannabinoid exposure is predictive of several adverse neonatal outcomes, most notably FGR and LBW. Physiological mechanisms that underly these abnormalities may also be associated with negative and persistent metabolic outcomes. The most recent data also suggests an association between *in utero* exposure to cannabinoids and cognitive, behavioral, and neuropsychiatric aberrations. Most notably, emerging evidence suggests an association between prenatal cannabinoid exposure and deficits in memory, attention, and learning. In addition, prenatal exposure is predictive of increased risk of depressive symptoms, prodromal symptoms of psychosis, and sleep disturbances. Importantly, these cognitive and neuropsychiatric aberrations appear early in development and are persistent into adolescence and early adulthood. Animal studies using cannabis constituents (Δ9–THC and CBD) have largely been consistent with the clinical observations, further providing possible mechanistic explanations. However, a consensus does not exist on many of these outcomes, largely owing to methodological limitations, some of which may be overcome. Notably, a shift from self-reporting to biological sampling would improve the quality of data collected in clinical settings, as would detailed analyses of the frequency of use and relative Δ9–THC dosing. Furthermore, considering some of the emergent

sex-dependent effects discussed in this review, it is possible that early inconsistencies were confounded by a lack of sex-specific analyses, which should be a major consideration for future investigations. For example, among animal studies covered in this review, 62% only considered male offspring (see **Supplementary Table 1**). With many cannabis-based products now on the market, it is also important to delineate the effect of chemical constituents in cannabis, such as the effects of Δ9–THC vs. CBD, as well as the method of consumption (e.g., inhalation vs. ingestion). Concurrent with this, more animal studies are needed to better establish causal links and plausible biological mechanisms. Importantly, growing evidence points to the critical role of prenatal factors such as the health of the placenta, the effects of intra-uterine growth restriction, and other pre-natal complications impacting the downstream risk of developing various neuropsychiatric disorders. There is thus an urgent need to better understand the mechanistic links between these prenatal developmental events, their impact upon neurodevelopmental pathology and risk factors and how exposure to cannabinoids might synergistically modulate these complex interrelationships.

AUTHOR CONTRIBUTIONS

MN researched and wrote the manuscript and created the tables. SL and DH provided intellectual input and edited and wrote the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.624275/full#supplementary-material

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METHODS
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Human Laboratory Models of Cannabis Use: Applications for Clinical and Translational Psychiatry Research

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Cannabis is increasingly used by individuals with mental health diagnoses and often purported to treat anxiety and various other psychiatric symptoms. Yet support for using cannabis as a psychiatric treatment is currently limited by a lack of evidence from rigorous placebo-controlled studies. While regulatory hurdles and other barriers make clinical trials of cannabis challenging to conduct, addiction researchers have decades of experience studying cannabis use in human laboratory models. These include methods to control cannabis administration, to delineate clinical and mechanistic aspects of cannabis use, and to evaluate potential treatment applications for cannabis and its constituents. In this paper, we review these human laboratory procedures and describe how each can be applied to study cannabis use in patients with psychiatric disorders. Because anxiety disorders are among the most common psychiatric illnesses affecting American adults, and anxiety relief is also the most commonly-reported reason for medicinal cannabis use, we focus particularly on applying human laboratory models to study cannabis effects in individuals with anxiety and related disorders. Finally, we discuss how these methods can be integrated to study cannabis effects in other psychiatric conditions and guide future research in this area.

Keywords: cannabis (marijuana), cannabinoids, psychiatric disorders, anxiety disorders, human laboratory research, clinical and translational research

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INTRODUCTION

Societal attitudes and public policy regarding cannabis use have shifted dramatically over the past two decades. Americans increasingly view cannabis as harmless (1), and as of December 2020, 36 states and the District of Columbia (DC) have legalized medicinal cannabis, while 15 states and DC permit recreational cannabis use. As acceptance of cannabis grows, more Americans are using, with 4.8 million more adults reporting near-daily cannabis use in 2018 compared to 2008 (2). Meanwhile, cannabis is purported to treat a variety of ailments. Despite limited evidence to support many of these claims, cannabis products are increasingly marketed as treatments for various medical conditions, including psychiatric disorders (3, 4). Thus, there is an urgent need to examine cannabis' purported mental health benefits and rigorously test its effects in patients with psychiatric disorders. In this paper, we describe how investigators can apply human laboratory methods to address these issues.

Why Study the Effects of Cannabis in Psychiatric Populations?

Americans increasingly use cannabis medicinally (i.e., with the intent to treat one or more symptoms) (5). Psychiatric symptoms including anxiety, depression, and stress are among the most common reasons for which patients report seeking medicinal cannabis (6, 7). Of 9,003 American adults who responded to a randomized, nationally-representative survey, 7% endorsed medicinal cannabis use, among whom 47 and 39%, respectively used cannabis to treat anxiety and depression (5). Among 2,774 American cannabis users in another survey, 13.6 and 12.7% reported using cannabis as a substitute for anxiolytics and antidepressant medication, respectively (8). Cannabis use to treat psychiatric symptoms may have further increased during the COVID-19 pandemic: Among 1,202 American medicinal cannabis users surveyed before and after the pandemic's onset, self-reported cannabis consumption increased by 91% more for those with mental health conditions vs. those without (9).

Although interest in, access to, and use of cannabis is increasing, surprisingly few studies have directly examined its effects in those with medical or psychiatric illness. As a result, there is limited evidence supporting using cannabis as a treatment. A 2017 report by the National Academies of Sciences, Engineering, and Medicine found sufficient evidence to support treating only two conditions with medicinal cannabinoids (i.e., cannabis constituents or analogs): Chronic pain (for which both oral cannabinoids and cannabis showed efficacy) and multiple sclerosis-related spasticity (for which only oral cannabinoids were effective) (10). The psychiatric literature is even sparser: A 2019 meta-analysis including 88 studies found insufficient evidence to support medicinal cannabis treatment of any psychiatric condition (7).

Despite this dearth of research, individuals with psychiatric illness increasingly use cannabis for both recreational and medicinal purposes (11). Yet lacking evidence of cannabis' effects on psychiatric symptoms, patients and clinicians have little information to guide decisions about how to use it. Whereas, FDA approval for medications requires release of detailed information about different doses, routes of administration, indications, and potential adverse effects, this information for a highly variable plant is largely unknown. Advertising from the now-billion-dollar cannabis industry has filled the information void and often includes claims about cannabis' purported psychiatric benefits (3, 12). Meanwhile, cannabis is a federally-illegal substance legalized by individual states, with scant consensus regarding how it should be used. For instance, a physician may recommend medicinal cannabis to a patient with PTSD in New York, but not in Iowa, while physicians in Colorado have discretion to recommend cannabis for any condition they determine it might help (including psychiatric illnesses) (13, 14). This creates a landscape that is bewildering to clinicians, who generally report feeling ill-equipped to make evidence-based recommendations about cannabis (15), and to patients, who may develop unrealistic expectations about what cannabis can and cannot do.

Regulatory and Scientific Challenges to Studying Cannabis Use in Human Volunteers

Clinical trials of cannabis are challenging to conduct in the US, partially due to cannabis' Schedule I labeling by the Drug Enforcement Agency (DEA). Researchers studying cannabis and other Schedule 1 substances must obtain special federal and state licensure (which can take months to years) following extensive monitoring and DEA-approved storage procedures (e.g., storing cannabis in a gun safe) (14). Thus, cannabis studies are typically limited to highly-specialized research environments. Moreover, current researchers may only use cannabis produced by the National Institute of Drug Abuse (NIDA) (14); in contrast, a range of cannabis products are sold in dispensaries and other commercial outlets or can be obtained illicitly (and may differ substantially from NIDA cannabis) (16).

In addition to regulatory factors, several scientific issues complicate studies of cannabis in human volunteers. Because cannabis is most often smoked or vaporized, different patterns of inhalation can lead to substantial variability in serum cannabinoid concentrations compared to, for example, intravenous administration (17, 18). Standardized methods to administer cannabis can minimize this variability. Smoking and vaporizing involve different preparation and delivery procedures than oral or intravenous administration (e.g., inserting plant material into a cigarette), requiring researchers to use different blinding techniques with these methods.

Cannabis' effects are susceptible to expectancy, such that individuals who anticipate receiving active cannabis but instead receive placebo nonetheless report experiencing cannabis-like effects (19). Psychiatric symptoms are also responsive to placebos, even those administered without deception (20). Thus, placebo control is essential for cannabis trials in psychiatric populations. Because many participants may be familiar with cannabis effects (for example, 16% of all Americans were estimated to have used cannabis in the past year in 2018) (2), placebo selection is also important to consider.

Dissecting the mechanistic properties and clinical effects of cannabis can also be difficult. Cannabis is pharmacologically diverse, containing over 140 unique chemical constituents ("phytocannabinoids"). Many phytocannabinoids are likely psychoactive, and the neurobiological mechanisms of even the two best-studied, Δ-9 tetrahydrocannabinol (THC) and cannabidiol (CBD), are incompletely understood (21). The properties of different cannabis varietals vary with their phytocannabinoid composition, the form, dose, and frequency in which they are administered, and the users' history of cannabinoid exposure (22). Disentangling the contributions of these factors can be difficult outside of controlled settings.

While few of cannabis' potential clinical benefits have been rigorously tested, its abuse potential has been well-documented (23). This poses an additional challenge to its study in individuals with psychiatric illnesses [who may be at increased risk for developing cannabis use disorder (CUD), among other adverse effects] (24). Investigators need to consider designs that can distinguish between cannabis' effects on psychiatric symptoms

(e.g., anxiolysis/anxiogenesis) and unrelated drug effects (e.g., intoxication), while also minimizing the risk that participants develop CUD or experience other cannabis-related harms.

Given the barriers involved in clinical research, cannabis' effects on psychiatric outcomes have mostly been examined through observational studies and surveys (7, 25, 26). These studies tend to rely on participants' retrospective self-reports of cannabis effects, which are subject to recall biases; in recruiting medicinal cannabis users (who by definition believe cannabis to be potentially helpful), they also involve selection bias. As noted above, both cannabis effects (19) and psychiatric symptoms (20) are influenced by expectancy. Given its pharmacologic diversity (22), accounting for the different effects of cannabis' various constituents (e.g., THC vs. CBD) is daunting even in controlled studies. In observational research, it is nearly impossible: Labeling of commercially-available cannabis products is frequently inaccurate (27, 28), state-run cannabis testing facilities have demonstrated systematic differences in the cannabinoid concentrations they report, and even experienced cannabis users have difficulty determining the THC/CBD content of the products they use from their subjective responses (29, 30). Further, cannabis that is smoked or vaporized vs. taken orally in tinctures or capsules will produce markedly different plasma cannabinoid concentrations (31).

Though observational research and surveys can be useful tools, their limitations make them insufficient to fully elucidate cannabis' clinical risks and benefits or its potential role in psychiatric treatment. Randomized, placebo-controlled trials remain the gold-standard tests of efficacy, yet only a few have examined cannabis' potential medicinal properties (of which only a subset involved patients with psychiatric disorders). Although small trials have tested psychiatric applications of synthetic cannabinoids (32) [e.g., nabilone, a synthetic THC analog that is approved by the US Food and Drug Administration (FDA) for treating cancer chemotherapy and HIV-related nausea and vomiting] and cannabinoid isolates (33) (e.g., various CBD preparations), recreational and medicinal users overwhelmingly ingest cannabinoids through inhaling smoked or vaporized cannabis flower (6, 16). While understanding cannabis' effects when used as it is most commonly in daily settings is critically important, a 2016 systematic review identified only one cannabis trial for any psychiatric indication (34). This open-label trial of smoked cannabis for PTSD lacked a placebo control or systematic method of cannabis administration (35). Since then, we have conducted two small placebo-controlled studies of smoked cannabis at our site: One tested its effects in individuals at high risk for psychotic disorders (36), and another tested its effects in patients with obsessive-compulsive disorder (OCD) (37).

Rationale for Using Human Laboratory Methods to Study Cannabis Use in Psychiatric Populations

Given the current political, social, medical, and legal climate, the public health need for controlled studies of cannabis effects in psychiatrically ill populations has never been more urgent. Whereas, psychiatric cannabis trials are nascent, addiction researchers have explored cannabis effects in human laboratory studies for decades (38). Human laboratory methods were developed to study problematic use of psychoactive drugs like cannabis and to identify new ways of treating individuals with substance use disorders. These procedures enable investigators to study and control methods of administration and to blind participants/investigators for rigorous testing of clinical effects. Researchers have also devised strategies to delineate factors contributing to the development and maintenance of CUD and other substance use disorders. Finally, the human laboratory has proved to be an efficient venue in which to screen for potential therapeutic effects of psychoactive substances like cannabis and cannabinoids before testing them in large-scale clinical trials. Herein, we review some of these human laboratory methods and describe how they could be applied to examine the effects of cannabis and cannabinoids in patients with psychiatric illnesses.

USING HUMAN LABORATORY METHODS TO STUDY THE EFFECTS OF CANNABIS AND CANNABINOIDS IN PSYCHIATRIC POPULATIONS

Overview: Substance use researchers have developed human laboratory methods to directly examine the effects of cannabis and its constituents. These include methods to control cannabis administration (e.g., dosing and blinding procedures), to delineate clinical and mechanistic aspects of cannabis use (e.g., intoxication and other acute effects, positive and negative reinforcement, dose-dependency, and tolerance), and to evaluate potential treatments (e.g., screening potential uses of cannabis in psychiatric treatment, testing treatments for comorbid psychiatric illness and CUD, and identifying cannabis-drug interactions). Below, we review these human laboratory procedures and describe their potential applications to explore cannabis effects in patients with psychiatric illnesses. Because anxiety disorders are among the most common psychiatric illnesses affecting American adults (39), and anxiety relief is also the most commonly-reported reason for medicinal cannabis use (5), we focus particularly on how human laboratory procedures could be applied to study cannabis effects in individuals with anxiety and related disorders. These procedures and associated applications are summarized in **Table 1**.

Methods to Control Cannabis Administration

Procedures to Control Dosing

Cued-smoking procedures have been developed to help standardize cannabis administration (64). Investigators provide participants a specific amount of cannabis containing known concentrations of constituents (e.g., THC, CBD), and then guide them through the process of smoking, controlling the duration of inhalation and the amount of time that smoke is held within the lungs (see **Figure 1** for details). Similar methods exist for controlled administration of vaporized (31, 65) and edible (31) cannabis formulations. Following cannabis administration,

TABLE 1 | Human laboratory procedures to model cannabis use and potential applications in patients with anxiety disorders.

Category	Model	Advantages	Challenges	Publications	Example applications in patients with anxiety disorders
Cannabis administration	Dosing	• Cued-dosing may improve standardization of cannabis delivery, while *ad-libitum* administration may reduce anxiogenic effects • Both procedures generate clinically-relevant effects • Methods exist to administer smoked, vaporized, and edible cannabis	• Cued-smoking may not reflect cannabis use in daily settings, while *ad-libitum* administration may increase variability in serum cannabinoid levels • Currently only NIDA-produced cannabis is permitted in human subjects research	Ramesh et al. (40) Haney et al. (41) Bidwell et al. (42)	-*Ad libitum* administration may generate sufficient cannabis exposure while mitigating potential anxiogenic effects from cued-dosing
	Blinding	• Reduce ability of investigators and participants to determine drug condition assignment • Limit observation of differences between laboratory-administered and naturalistically-used cannabis	• Participants may still detect psychoactive properties of cannabis • Difficult to design active controls	Chait et al. (43, 44) Kirk et al. (45) Metrik et al. (19)	- Careful attention to participant selection (e.g., excluding heavy cannabis users) and instructions (e.g., notifying participants that they will smoke cannabis containing a range of phytocannabinoids contents with varied effects on anxiety) may limit blinding failure
Clinical and mechanistic aspects of cannabis use	Intoxication & acute effects	• Measuring acute response to cannabis administration can help to establish a timecourse for cannabis effects • Can incorporate subjective (e.g., self-report) and objective (e.g., computerized cognitive tasks) measures to track outcomes of interest	• Effects may vary based on prior exposure to cannabis • Response may differ with long-term or repeated administration 3. Few current options for measuring rapid changes in psychiatric symptoms	Hart et al. (46) Vadhan et al. (47) Ramaekers et al. (73)	- Incorporating a visual analog scale to probe for rapid changes in anxiety symptoms
	Positive reinforcement & reward	1. Self-administration paradigms can model cannabis use to increase positive affect 2. Can examine reinforcement differences based on cannabinoid content and relative to other reward outcomes (e.g., food, money)	1. May be difficult to disentangle increased positive affect vs. decreased negative affect	Haney et al. (48) Hart et al. (49) Cooper and Haney (50)	- Comparing cannabis self-administration among anxious and non-anxious participants - Comparing self-administration of cannabis vs. benzodiazepines in anxious participants
	Negative reinforcement & withdrawal	1. Withdrawal/abstinence paradigms can model cannabis use to mitigate negative affect • Abstinence paradigms may be less logistically/ethically challenging to conduct than cannabis administration paradigms • Can also incorporate tasks to measure intoxication effects on negative affect	• Often requires inpatient admission 2. Differentiating negative affect related to withdrawal vs. psychopathology may be difficult	Metrik et al. (51) Hefner et al. (52, 53) Haney et al. (54)	- Assessing cannabis effects on tasks indexing anxiety states (e.g., the NPU task, which indexes startle response to predictable vs. unpredictable threat) in cannabis users with anxiety disorders. - Could assess participants' response to cannabis intoxication or compare effects of continued use vs. abstinence
	Dose-dependence & Tolerance	• Repeated-administration designs can identify tolerance to physiological and intoxication effects • Can also examine effects of different doses/concentrations	• Some acute cannabis effects (e.g., on impulse control, etc.) may persist even with repeated administration – tolerance to psychotropic effects is not clearly established	Haney et al. (55) Hart et al. (46) Ramaekers et al. (73)	- After first showing acute cannabis effects on psychiatrically-relevant outcomes, studies can explore whether the magnitude of these effects declines with continued administration - Exploring for dose-dependency by comparing cannabis varietals with varied concentration of THC and other phytocannabinoids

(Continued)

TABLE 1 | Continued

Category	Model	Advantages	Challenges	Publications	Example applications in patients with anxiety disorders
Potential pharmacological treatments	Therapeutic applications of cannabis	• Can address the critical need for placebo-controlled studies of cannabis effects • Within-subjects, repeated-measures designs allow trials to be conducted efficiently with adequate statistical power even at modest sample sizes • Human laboratory as a translational bridge to screen promising therapeutic applications for cannabis prior to investing in large-scale clinical trials • Allow testing of cannabis' behavioral, physiological, psychological, and neurocognitive targets, which is aligned with current NIMH initiatives to identify objective measures of psychopathology and enhance clinical trials	• Controlled laboratory environments may not reflect cannabis use in naturalistic settings • Researchers are currently restricted to using NIDA-produced cannabis • Participants in laboratory studies may differ from general psychiatric populations 4. Different challenges depending on illness being studied (e.g., risk for psychosis in individuals with bipolar disorder)	Vadhan et al. (36) Kayser et al. (37)	- Assessing acute effects of cannabis in individuals with anxiety disorders by first administering a single dose of cannabis followed by repeated assessments of self-reported anxiety, cardiovascular measures (e.g., heart rate), and threat response - Using within-subjects designs to compare the effects of different cannabis varietals (e.g., high-THC, high-CBD) vs. placebo
	Treatments for comorbid CUD + psychiatric illness	• Treatments for shared symptoms of CUD and psychiatric disorders can be evaluated in models of relapse or abstinence from cannabis use • Examining discrete outcomes related to cannabis use (e.g., withdrawal, relapse) and psychopathology (e.g., symptom self-report) can clarify the mechanistic basis for treatment effects	• Disentangling effects of CUD vs. psychopathology may be challenging (e.g., self-reported anxiety due to GAD vs. cannabis withdrawal)	Haney et al. (41, 56–59) Herrmann et al. (60)	- Assessing medications to treat symptoms of GAD and cannabis withdrawal (e.g., anxiety, irritability, restlessness, insomnia) in cannabis users with GAD - To clarify mechanism for any observed medication effects, outcomes to be examined might include. Cannabis self-administration, anxiety self-report, and threat response.
	Drug-drug interactions with cannabis and/or cannabinoids	• Cannabis contains >140 phytocannabinoids and thus could interact with many commonly-used medications • Laboratory procedures can screen for such interactions under controlled conditions • Can also determine whether preclinical evidence for cannabis-drug interactions replicates in human subjects	• Potential drug-drug interactions for the vast majority of phytocannabinoids are largely unexplored even in preclinical studies	Hartman et al. (61) Gaston et al. (62) Alsherbiny and Li (63)	- Assessing serum levels of anxiolytic medications (e.g., SSRIs) following coadministration with cannabis (or cannabinoids)

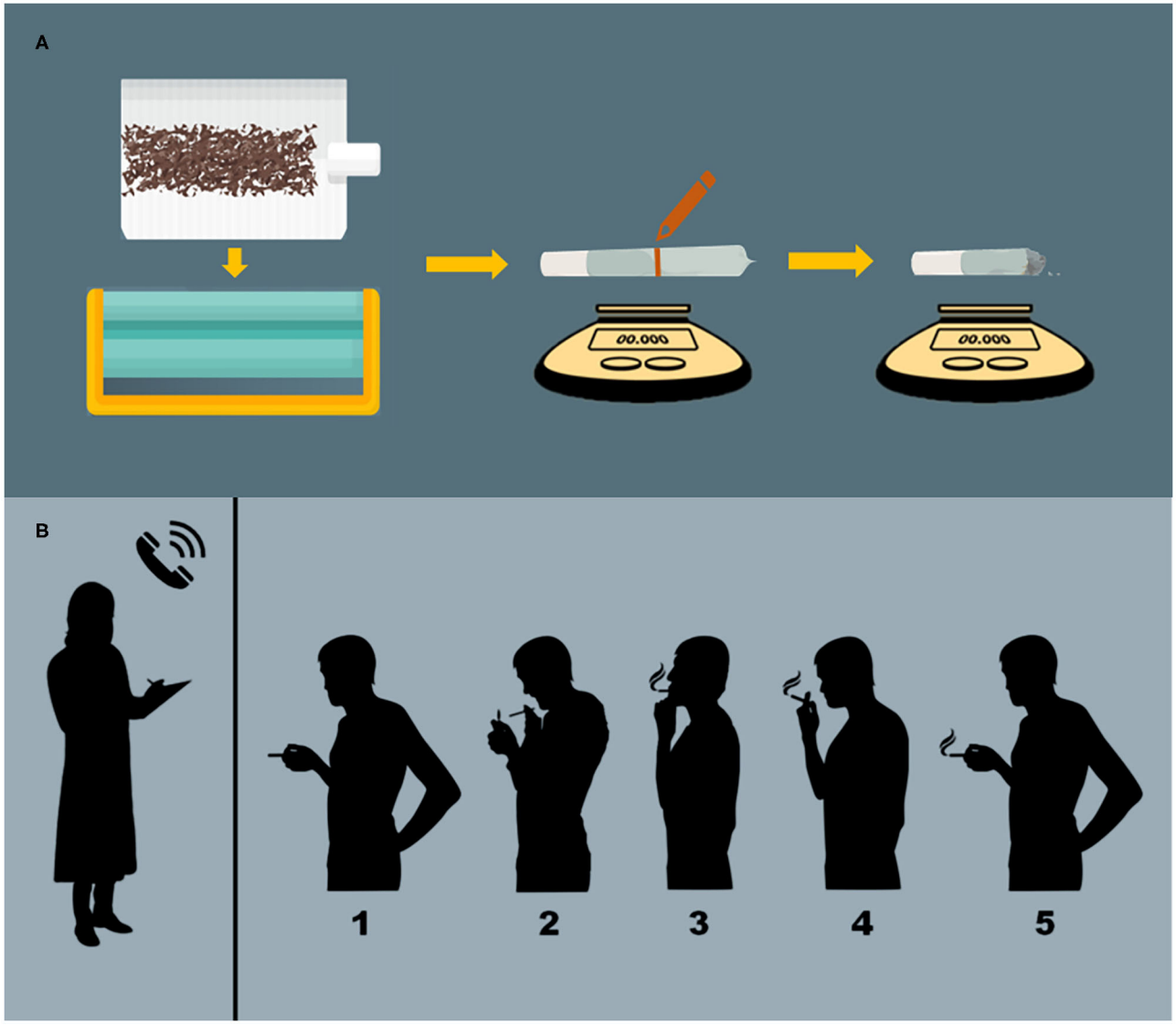

FIGURE 1 | Administration procedures. **(A)** Preparation of cannabis cigarettes. Cannabis cigarettes are machine-rolled using cigarette paper and then inserted into a plastic cigarette holder. A line is drawn at the half-way point and the participant is instructed to smoke 50% of the cigarette. Cannabis consumption is verified via pre- and post-administration weighing of cigarettes. **(B)** Cued-smoking procedure. From a separate room with a two-way mirror, an investigator (who has no other contact with participants) guides participants through cued-smoking procedures. (1) The participant is presented with the cannabis cigarette, and then instructed to (2) "Prepare" (light the cigarette and prepare to smoke), (3) "Inhale" (5 s), (4) "Hold smoke in lungs" (10 s), and (5) "Exhale." This cycle is repeated, allowing a 40 s interval between puffs, until 50% of the cigarette is pyrolized.

participants' subjective, physiological, and/or neurocognitive responses can be measured at precise timepoints (38).

Though potentially improving standardization, cued-smoking procedures may not reflect how cannabis is used in daily settings. Moreover, asking participants to smoke a specific percentage of a cannabis cigarette (rather than allowing them to titrate to their desired level) may induce discomfort or anxiety in some individuals if, for example, they are required to smoke more cannabis than they are comfortable smoking (51). Some studies have accounted for such effects by instructing participants to smoke *ad libitum* over the course of a predefined time period (e.g., 10 min) (42, 66). A*d libitum* cannabis administration may increase variability in serum cannabinoid concentrations, but recent studies suggest it nonetheless yields clinically-relevant effects (42, 66–68). Thus, in a study of patients with anxiety disorders, *ad libitum* procedures may generate sufficient cannabis exposure while also mitigating potential anxiogenic effects due to administration procedures (rather than cannabis itself) that might occur with cued-smoking.

Despite attempts to standardize administration procedures, cannabis smokers adjust their inhalation patterns as a function of cannabinoid content (i.e., decrease inhalation as THC content

increases, and vice versa) (40, 69). As a result, both cued-smoking and *ad libitum* administration yield relatively consistent serum cannabinoid concentrations, even when accounting for differences in potency (i.e., THC content) (69). Nonetheless, participants experience clinically-relevant effects when guided through these smoking procedures. Indeed, even heavy users who are tolerant to cannabis will become intoxicated from controlled administration of low-potency cannabis in the human laboratory (41).

Procedures to Improve Blinding

Placebo-controlled trials assume that participants and investigators are blinded to drug conditions (i.e., that inactive and active agents are indistinguishable). Blinding is critical in cannabis research because cannabis users experience significant expectancy effects when exposed to cannabis-related cues (e.g., cigarette appearance and smell, the act of smoking) (43, 45, 70), and also report subjective cannabis-like effects when they anticipate receiving active cannabis but instead receive placebo (19). Moreover, participants' observation of differences between laboratory-administered cannabis and the cannabis they use outside of the lab may influence expectancy (71). As described above, psychiatric symptoms are also particularly sensitive to expectancy effects; thus, adequate blinding is essential to studying cannabis effects in psychiatric illness. Fortunately, human laboratory researchers have developed extensive procedures to improve blinding to cannabis dosing conditions (44).

In the cannabis administration procedures outlined above, blinding is maintained through the following methods (detailed in **Figure 1**): (36, 37, 41). First, cigarettes are machine-rolled using cigarette paper. They are then inserted into a plastic cigarette holder and a line is drawn at the half-way point, after which the cigarette is presented to the participant. The participant is then guided through the smoking procedure until 50% of the cigarette is smoked (verified by pyrolization to the half-way mark on the cigarette). Smoking only half of a cigarette prevents participants and investigators from seeing the color of its contents (which might vary across conditions or differ from the cannabis participants use in daily settings) and masks the moisture content of the cigarette (which affects burn time and may be higher in placebo vs. active cannabis). Smoking through a plastic cigarette holder also prevents participants from squeezing and possibly occluding the end of the cigarette with their lips, and ensures more consistent puff-to-puff delivery of smoke components, which vary (often increase) with successive puffs (44). Once participants have smoked to the 50% mark, consumption can also be verified via pre- and post-administration weighing of cigarettes (41).

Another approach to the blinding problem is to instruct participants that they will smoke cannabis containing a wide range of THC and other cannabinoids, some of which are intoxicating and others which are not, and ask them to guess their treatment assignment after study completion (72). Across a variety of human laboratory studies (19, 69), individuals receiving placebo cannabis often guess that they instead received a low-potency (but still active) varietal, suggesting the presence of expectancy effects. Investigators can also assess participants' self-report of psychological and physiological effects from active vs. placebo cannabis (19, 40). Other proposed approaches have included recruiting cannabis-naïve participants, which may improve blinding but also potentially increase risk for addiction and other adverse effects (e.g., panic attacks), or using active controls, which may be challenging in that it is unclear which substance suitably mimics the effects of cannabis (euphoria, dry mouth, tachycardia, etc.) without affecting other relevant outcomes (71). Finally, using within-subjects designs, investigators can compare different cannabis varietals with varied concentrations of THC and other cannabinoids (36, 37) while also reducing participants' ability to determine their assigned condition by increasing the range of phytocannabinoids concentrations they could possibly receive.

The blinding approaches above could easily be applied to study how cannabis affects individuals with anxiety disorders. That said, the instructions participants receive should be designed carefully to limit potential expectancy effects on self-reported anxiety: For example, investigators may inform patients that they will smoke cannabis with different concentrations of THC/CBD (rather than active cannabis vs. placebo), which may have a range of effects on anxiety (rather than being anxiolytic or anxiogenic). Excluding heavy cannabis users (e.g., weekly or greater) may reduce the chances that experienced participants guess their assigned condition (in addition to mitigating tolerance effects); to limit risk for adverse cannabis effects, researchers could recruit participants with at least some prior experience using cannabis without negative effects (e.g., >1 lifetime use without experiencing a panic attack).

Methods to Dissect Clinical and Mechanistic Aspects of Cannabis Use

Intoxication and Other Acute Effects

Acute cannabis effects can be examined in laboratory studies by obtaining self-reports, physiological assessments, and/or neurocognitive tests at specific intervals following cannabis administration; these methods also permit exploration of cannabis' acute effects on psychiatric outcomes. Cannabis studies typically ask participants to self-report ratings of intoxication, including how "high" they feel, cannabis "liking," and "good/bad effect." Because THC produces dose-dependent increases in heart rate, researchers often integrate serial physiological assessments to establish a timeline for acute cannabis effects. Laboratory studies have also included repeated self-report assessments to probe acute changes in psychiatric symptoms: (36, 37). For example, patients with OCD in our cannabis trial were asked to complete standardized scales of obsessions, compulsions, and anxiety following cannabis administration (37). Other studies have used computerized cognitive tasks [administered once (46) or serially (47)] or obtained neuroimaging assessments (73) to examine acute cannabis effects on neurocognitive outcomes.

Selecting appropriate self-report instruments may be challenging for psychiatry researchers, since many validated scales measure symptoms over long-term (i.e., weeks to

months) rather than rapid timeframes (i.e., minutes to hours) (74). While better ways to assess acute changes in psychiatric symptoms are needed, pending their development, studies of rapid-acting treatments (e.g., ketamine) often use a simple visual analog scale (VAS) to identify symptomatic changes (75, 76). In the above laboratory study in patients with OCD, we used a VAS to explore patients' self-report of change in obsessions and compulsions (on a scale from 1 to 10); (37) similar measures could easily be developed to explore cannabis-related symptomatic changes in patients with anxiety or other psychiatric disorders.

Positive and Negative Reinforcement

Behavioral pharmacology studies in non-treatment seeking cannabis smokers demonstrate that cannabis is positively reinforcing: Given the option to self-administer different cannabis varietals in a laboratory setting, participants will administer THC-containing cannabis more often than cannabis containing minimal THC (50). Depending on THC content, participants in these paradigms will also choose to receive THC-containing cannabis over non-drug alternatives like money (49) or a preferred food (48). The *incentive-sensitization* model describes how positive reinforcement may contribute to increased cannabis use among those with psychiatric illness: Individuals who associate cannabis with pleasure develop greater motivational salience toward cannabis-related cues, which elicits more approach behaviors and attentional bias toward cannabis cues that ultimately increase the likelihood of further cannabis use (77). Several psychiatric conditions including attention-deficit-hyperactivity disorder (ADHD) involve deficits in motivation and attention, reflecting dysfunction in reward-related (particularly dopaminergic) neural circuits (78, 79). Individuals with such deficits may be more susceptible to positive reinforcement from cannabis, which is consistent with epidemiological data supporting higher rates of cannabis use for those with untreated ADHD than in the general population (80).

To date, most laboratory investigations of cannabis' capacity for positive reinforcement have been in cannabis users or adults with CUD. However, self-administration paradigms could also be used to delineate cannabis-related positive reinforcement effects in participants with psychiatric disorders. One example would be for researchers to compare self-administration of cannabis among adults with anxiety disorders and controls matched for their patterns of cannabis use. Another would be to offer anxious participants the choice to receive either cannabis or anxiolytic medications known to be positively-reinforcing (e.g., benzodiazepines) (81).

There is also substantial evidence that cannabis is negatively reinforcing, meaning that individuals use it to escape or reduce the effects of aversive states (e.g., negative affect, withdrawal) (82). Laboratory models of cannabis-associated negative reinforcement typically focus on withdrawal states, admitting participants to an inpatient unit where their access to cannabis is controlled and/or stopped completely (54, 83) and then assessing symptoms of cannabis withdrawal (e.g., disrupted sleep, negative mood) and self-administration. These procedures also have identified differences in cognitive (e.g., reward valuation) (52) and physiological processes (threat response) (53) between cannabis users and controls. Specifically, compared to non-users, heavy cannabis users who abstained from cannabis for 3 days showed greater uncertainty aversion on a reward valuation task (52), while both abstinent and non-abstinent cannabis users had increased startle responses to unpredictable threat (a physiological marker of anxiety states) (53).

According to the *affect-motivational model*, negative reinforcement drives cannabis use by some individuals with affective psychopathology (e.g., depression/anxiety disorders), who may use cannabis situationally to attenuate affective symptoms (82). Supporting this idea, both depressive and anxiety disorders are linked to higher-than-average rates of cannabis use (82), and alleviating depression/anxiety symptoms is among the most commonly-cited reasons for which individuals seek medicinal cannabis treatment (5, 84). Moreover, preliminary neuroimaging data in both cannabis users (85) and non-cannabis using healthy volunteers (86, 87) suggest that THC acutely reduces functional activity in brain regions involved in emotional processing, particularly when evaluating negative face emotions.

Laboratory probes for negative reinforcement could test whether cannabis use alleviates symptoms or other aversive states in individuals with specific psychiatric diagnoses. Investigators might do this by assessing for differences in disease-relevant outcomes (e.g., symptom self-report, physiological measures, neurocognitive task performance) under conditions of continued use vs. abstinence, or following active vs. placebo cannabis administration. In the case of anxiety disorders, the neutral/predictable/unpredictable shock (NPU) task offers an example of an outcome that is sensitive to both disease- and cannabis-related effects. The NPU task, which indexes startle response to unpredictable vs. predictable threat, can discriminate between anxiety and fear states (88), has been used to screen for the effects of anxiolytic medications (89), and has identified effects related to cannabis withdrawal along with differences between cannabis users and controls (53). The task could easily integrate into laboratory models of intoxication or withdrawal, providing a powerful tool to evaluate for cannabis-related effects on anxiety.

Dose-Dependency and Tolerance

Dose-dependent cannabis effects have also been identified using human laboratory procedures (40, 90). These studies consistently find that cardiovascular outcomes and (to a lesser extent) self-rated subjective responses are sensitive to variation in THC content (40). Dose-response relationships for subjective responses have been more difficult to establish, possibly due to stronger influence of expectancy effects on self-report outcomes. Performance on error-monitoring tasks (e.g., the Flanker task) and other neurocognitive measures has also been shown to vary with THC dose (90).

Tolerance to the effects of THC-containing cannabis develops rapidly over the course of a few days. Cannabis users who were admitted to an inpatient unit where they received smoked cannabis initially reported acute increases

in euphoria and intoxication (e.g., "high," "good drug effect"), but the magnitude of these effects declined over several days of repeated administration. Moreover, tolerance developed dose-dependently (i.e., was greater when high-THC cannabis was administered compared to low-THC) (55). Tolerance to cannabis' physiological effects (e.g., tachycardia) developed dose-dependently over a similar timeframe in other studies (46). In contrast, cannabis' effects on neurocognitive functions like impulse control may persist even with sustained administration (91).

Similar designs could help to determine whether dose-dependency or tolerance moderate cannabis effects on psychiatrically-relevant outcomes. One strategy would be to recruit individuals with anxiety disorders to receive several cannabis varietals with varied THC content to determine whether THC dose moderates self-reported anxiety, blood pressure/heart rate, and or cognitive/physiological measures (e.g., the NPU task). If dose-dependent THC effects are identified, investigators could then assess whether tolerance develops following repeated administration. Establishing whether dose-dependency or tolerance occur will be critical in determining cannabis' potential role in treating anxiety or other psychiatric symptoms.

Methods to Evaluate Pharmacological Treatments

Laboratory procedures already used to screen for CUD treatments can also be applied to study cannabis' role in psychiatric treatment, specifically by screening for potential uses of cannabis to treat symptoms of psychiatric disorders, evaluating medications to treat comorbid psychiatric and CUD symptoms, and assessing for cannabis-drug interactions. Examples of each application are provided below.

Potential Uses of Cannabis to Treat Psychiatric Illness

The human laboratory can serve as a translational bridge to move promising preclinical findings into clinical studies of cannabis. In this regard, a critical use for laboratory paradigms is to test the safety, tolerability, and clinical effects of cannabis in psychiatrically ill individuals. Findings from observational cannabis studies are often difficult to apply in real-world clinical scenarios because (as described above) these designs rarely capture the types of cannabis participants use, or how they ingest it. In contrast, human laboratory procedures permit delivery of precise amounts of cannabis in various forms (e.g., smoked, vaporized, or edible) and containing known phytocannabinoid concentrations. As a result, investigators are able to more accurately determine how the dose, formulation, and contents of cannabis relate to its clinical effects.

Human laboratory paradigms can also be used to validate cannabinoids' hypothesized targets. For example, investigators might test how cannabis acutely modulates brain function using task-based fMRI, or alters cognitive or physiological outcomes during paradigms like the NPU task. Evidence that cannabis meaningfully changes these outcomes could inform mechanistic understanding of its effects in psychiatric illness and may suggest potential treatment applications for further testing.

Finally, the human laboratory is an ideal venue for conducting preliminary tests of cannabis' efficacy as a psychiatric treatment. By incorporating placebo control and rigorous blinding procedures, laboratory paradigms are better able to count for expectancy effects than observational studies or surveys. Compared to clinical trials, human laboratory studies are also faster, cost less, and enable tighter control over potential confounds. Many use within-subjects designs that can achieve adequate statistical power with a smaller number of participants, facilitating testing of clinical effects, mechanistic hypotheses, and potential response moderators [e.g., age (92), gender (67, 93), genetics (94, 95), psychiatric history (96), and prior cannabis exposure (97)]. Laboratory models can thus function as a key intermediary step between preclinical research and clinical trials, rapidly generating data about the odds that cannabis treatment will succeed, which would then guide decisions about the utility of large-scale, resource-intensive clinical trials (98).

Treatments for Comorbid Psychiatric Disorders and CUD

Psychiatric comorbidity is common among adults with CUD, and conversely, psychiatrically-ill individuals are at greater-than-average risk for CUD (24). Though few laboratory studies have explored treatments for these combined conditions, CUD-relevant outcomes have been modeled extensively in the laboratory. These include relapse, operationally defined in the human laboratory as self-administration of cannabis following a period of abstinence. Though it would be unethical to offer cannabis to individuals seeking treatment for cannabis use, relapse can be modeled in non-treatment seeking cannabis users. Participants are typically admitted to an inpatient unit where they remain abstinent for several days. Then, they are given the choice to purchase individual puffs of a cannabis cigarette. Money not spent on cannabis self-administration is given to participants at study end. The initial puff, which reflects "relapse" to cannabis use, carries the greatest cost, while the cost of subsequent puffs decreases (56). Around 50% of participants in studies following these procedures will choose to "relapse"; (38) thus, investigators can explore how treatments influence the decision to resume cannabis use (56–59). Using similar methods, future studies might explore whether medications (e.g., SSRIs) moderate risk for cannabis relapse among individuals with anxiety disorders and CUD.

Cannabis self-administration models have also been used to test medications targeting symptoms of cannabis withdrawal. In one such paradigm, daily cannabis users, abstinent from cannabis for several days, were treated with nabilone at either 6 mg or 8 mg/day vs. placebo. Nabilone improved withdrawal-associated irritability and insomnia while significantly reducing the choice to pay money to self-administer cannabis following abstinence (i.e., a laboratory model of relapse) (56). A follow-up study found that adding zolpidem to nabilone more robustly targeted insomnia, with this combination yielding improved negative mood, anorexia, and insomnia while decreasing cannabis relapse rates compared to placebo or zolpidem alone (60).

Both cannabis withdrawal and generalized anxiety disorder (GAD) involve symptoms of anxiety, irritability, restlessness, and insomnia, which may lead those with GAD to experience withdrawal symptoms more frequently or intensely, increasing their risk for continued cannabis use and relapse (99). Thus, using similar laboratory methods, investigators could examine medications or psychotherapies hypothesized to effectively treat these shared symptoms.

Finally, laboratory researchers have evaluated potential treatments to help individuals with CUD achieve abstinence. A straightforward approach used in many studies is to provide non-treatment-seeking cannabis users with either a medication or placebo, and then assess for between-group differences in cannabis self-administration (i.e., whether cannabis use is maintained, reduced, or stopped) (41, 56). Researchers have also used this procedure to explore the abstinence-promoting effects of contingency management paradigms (which offer participants monetary incentives to abstain from cannabis) (100) and cognitive behavioral therapy (CBT) (101). One preliminary study found that a modified form of CBT targeting both anxiety and CUD symptoms reduced self-reported anxiety and cannabis use among individuals with CUD and anxiety disorders; (102) future studies might examine whether this intervention moderates laboratory models of abstinence in this population.

Drug-Drug Interactions Between Cannabis and Psychotropic Medications

Two substances administered simultaneously may interact by pharmacodynamic (i.e., affecting the same receptor or target) and/or pharmacokinetic (i.e., affecting absorption, distribution, metabolism, or excretion) mechanisms. The most commonly reported drug-drug interactions involve pharmacokinetic changes to the activity of cytochrome P450 (CYP450) enzymes, leading to altered drug metabolism. With over 140 phytocannabinoid constitutents (103), cannabis can potentially interact with a range of medications. Animal studies suggest that THC and CBD be substrates for and inducers/inhibitors of CYP450 enzymes (63). With a diverse array of targets including $5HT_{1A}$ receptors, CBD also has a variety of potential pharmacodynamic interactions with psychotropic drugs (104).

While not all drug-drug interactions identified in animal models are clinically relevant, human trials of both THC and CBD have shown that they interact with common medications. In patients with epilepsy, co-administration of CBD modified serum levels of various antileptics including topiramate, clobazam, and zonisamide (62). Conversely, in adult cannabis users, alcohol increased serum THC levels when co-administered with cannabis (61). Preliminary studies also suggest that cannabis and its constituents can interact with warfarin, oxymorphone, disulfiram, pentobarbital, and cocaine, among other agents (63). Interactions between cannabis/cannabinoids and most psychotropic medications (including anxiolytics) have not been rigorously tested. The human laboratory may be an ideal venue to assess for these potential interactions under controlled conditions.

Integrating Human Laboratory Procedures to Study Cannabis Effects in Psychiatric Illness: Example From a Study in Adults With OCD

Our human laboratory study of smoked cannabis in adults with OCD offers one example of how these paradigms could be applied to screen for therapeutic cannabis effects and inform future clinical and translational research (37). Considering preclinical evidence that cannabinoids affect key cognitive processes and neural circuits implicated in OCD (105), along with anecdotal reports from our clinic patients who suggested that cannabis relieved their symptoms, we conducted a randomized, placebo-controlled, within-subjects study. Twelve adult participants with OCD received three cannabis varietals over the course of three laboratory sessions: High-THC (7.0% THC/0.18% CBD), high-CBD (0.4% THC/10.4% CBD), and placebo (0% THC/CBD). Cannabis was administered using cued-smoking procedures, and serial measurements of OCD symptoms, state anxiety, intoxication, and cardiovascular measures were obtained over 3 h. We found that OCD symptoms and state anxiety decreased immediately following cannabis administration in all three conditions. However, there were no differences in OCD symptoms as a function of cannabis condition. Further, placebo cannabis yielded greater reductions in state anxiety than either active varietal. High-THC cannabis significantly increased heart rate and self-reported intoxication compared to both high-CBD and placebo, demonstrating that the cannabis exposure was sufficient to produce physiological and subjective effects.

This human laboratory study integrated several of the procedures reviewed above, including cued-smoking and blinding methods, self-report scales measuring psychiatric symptoms and intoxication, and physiological assessments. Findings have important clinical, public health, and research implications. Our data suggest that smoked cannabis may have little short-term benefit to individuals with OCD, which would argue against clinical use of cannabis as an acute OCD treatment, inclusion of OCD among the indications for physician-recommended cannabis, or conduct of large-scale clinical trials of smoked cannabis for the acute treatment OCD. Alternatively, finding acute benefits from active cannabis over placebo would have supported further study of its potential clinical utility in OCD: This might have included laboratory examinations of the potential risks and benefits of longer-term cannabis use in OCD (i.e., repeat administration over days to weeks), larger-scale trials assessing its acute efficacy for treating OCD symptoms, or mechanistic studies exploring the basis for the preliminary clinical effects that were observed. Because our preliminary study did not support these larger trials, we were able to quickly move on to pursue alternative research directions.

We then asked a different empirical question: Can THC facilitation of extinction improve the efficacy of existing therapeutic approaches? In a small pilot trial, we tested the effects of 4 weeks of daily treatment with nabilone (an FDA-approved synthetic THC analog) in patients with OCD, and found that nabilone had little effect on OCD symptoms as monotherapy, but appeared to enhance the effects of exposure-based psychotherapy

when both were combined (106). This finding was consistent with animal (107, 108) and human neuroimaging data (109–112) suggesting that THC facilitates extinction learning, which is thought to occur during exposure treatment for OCD (113). Thus, THC may have therapeutic benefit to individuals with OCD when paired with exposure treatment.

Based on these findings, in an upcoming fMRI study, we will test the hypothesis that nabilone facilitates extinction learning by impacting relevant brain circuitry. In a separate study, we will also assess whether anxious individuals respond similarly to those with OCD following acute cannabis challenge (i.e., experience smaller anxiety reductions with active cannabis vs. placebo). Using a similar human laboratory design, we will examine the acute effects of smoked cannabis on self-reported anxiety, physiological response to threat, and intoxication in adults with anxiety disorders and high trait anxiety. These novel research directions demonstrate how human laboratory paradigms can guide clinical and translational research involving the effects of cannabis and cannabinoids in psychiatric illness, whether results are positive or negative.

DISCUSSION

Human laboratory models have been used to understand why individuals use cannabis, to define factors that may contribute to CUD, and to test potential treatments for problematic cannabis use. Applying these procedures can also help elucidate the relationship between cannabis use and psychiatric disorders. Laboratory methods permit controlled administration of cannabis under blinded conditions and assessment of interactions between psychiatric symptoms and discrete cannabis-related outcomes (e.g., intoxication, positive and negative reinforcement, dose-dependency, and tolerance). Finally, the human laboratory can be a powerful translational venue in which to screen potential applications of cannabis or its constituents to treat psychiatric symptoms, evaluate treatments for comorbid psychiatric illness and CUD, and identify cannabis-drug interactions.

A key strength of laboratory models is that they can resolve the acute effects of cannabis on discrete behavioral (e.g., self-administration, choice of non-cannabis rewards), psychological (i.e., self-reported or clinically-assessed symptoms), physiological (e.g., cardiovascular and pharmacokinetic measures), and neurocognitive outcomes (e.g., performance on computerized cognitive tasks, neuroimaging assessment). Laboratory researchers can explore endpoints that are directly related to cannabis use (e.g., models of cannabis relapse) and those that are not (e.g., performance on a social-stress paradigm) (114), and can incorporate both subjective (i.e., self-report) and objective (e.g., physiological) assessments. This ability to test cannabis effects across various levels of analysis is consistent with the US National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) (115) and other initiatives aimed at developing more objective measurements of psychopathology (116). Moreover, by incorporating fMRI and other neurobiological measures (73), laboratory models might reveal targets to index cannabis effects that could then be explored in future treatment studies. Thus, the goals and designs of human laboratory research are also well-matched to experimental medicine approaches to psychiatric treatment development (117).

Of course, human laboratory research is not without limitations. First, while tight control over various confounding factors is a key strength of laboratory paradigms, this may also limit their generalizability, as real-world settings are rarely so well-regulated. Whether laboratory studies accurately capture cannabis effects on psychopathology or predict medication efficacy also depends on the chosen design and outcome measures. For example, a study of cannabis effects in specific phobia that does not incorporate symptom provocations may fail to detect an anxiolytic signal even when one exists (since patients with specific phobia typically have minimal anxiety in the absence of phobic triggers). In contrast, a finding that cannabis acutely reduces scores on the Depression, Anxiety, and Stress Scale; DASS) in patients with GAD may lead investigators to conclude that cannabis has anxiolytic effects, when in fact participants misinterpreted reduced stress and tension as reflecting anxiety relief (as prior studies in cannabis users suggest they may do) (118).

Second, participant selection is critical to consider given that the risks of cannabis are different for individuals with different psychiatric disorders: Adults with GAD may be at relatively low risk from participating in a study modeling acute effects from smoking one cannabis cigarette, but the same paradigm would involve different risks and ethical concerns in children with GAD (e.g., increased risk for psychosis) or adults with panic disorder (e.g., panic attacks). Even among participants with the same disorder, individual factors like age (92), gender (67, 93), or genetics (94, 95) may influence the response to cannabis and need to be considered when designing studies. Beyond participant selection, volunteers for laboratory studies may differ from general psychiatric populations in important ways: For example, they tend to have fewer medical comorbidities in order to pass inclusion criteria allowing cannabis to be safely administered (98). Moreover, individuals motivated to participate in a cannabis study presumably have neutral or positive expectations about its effects, which could positively bias study results.

Finally, in the US, only cannabis produced by NIDA can be used in human subjects research (14). Yet the available NIDA cannabis varietals differ substantially in their phytocannabinoid contents compared to cannabis available in the community through both legal (119) and illicit means (120). In particular, THC concentrations on average are lower with NIDA cannabis, which has raised concerns about the generalizability of research involving NIDA preparations. However, there are dozens of studies showing that daily, heavy cannabis users (who are presumably tolerant to THC) become intoxicated and show reliable increases in heart rate after smoking NIDA cannabis (41). Thus, despite differences in cannabinoid content between NIDA and community-obtained cannabis, human laboratory models may nonetheless provide clinically-relevant information about cannabis effects in human subjects.

CONCLUSION

In summary, human laboratory procedures have a rich history in the field of substance use research. Laboratory methods can also be applied to examine how psychopathology relates to cannabis use, clarify the risks and benefits of cannabis use to individuals with psychiatric disorders, and screen for potential applications of cannabis in psychiatric treatment. Exactly which designs and endpoints best capture specific psychopathologies remains to be determined and should be explored. In addition, while placebo-controlled studies in the human laboratory may provide the necessary groundwork to justify future cannabis trials, further research is needed to verify that promising findings from laboratory models of cannabis treatment are indeed replicable in psychiatric clinical trials. Nonetheless, these laboratory models are powerful tools that can address the increasingly critical need to understand the relationship between cannabis use and psychiatric illness. By improving understanding of cannabis' risks, benefits, and potential treatment applications for patients with psychiatric disorders, laboratory models can enhance the way we conceptualize, diagnose, and treat individuals who suffer from both anxiety and other mental health disorders as well as problematic cannabis use.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

RK, MH, and HS contributed to the conceptual framework, literature review, methodological design, and manuscript writing and preparation. All authors contributed to the article and approved the submitted version.

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Prospects for the Use of Cannabinoids in Psychiatric Disorders

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Increasing evidence suggests an essential role of the endocannabinoid system in modulating cognitive abilities, mood, stress, and sleep. The psychoactive effects of cannabis are described as euphoric, calming, anxiolytic, and sleep-inducing and positively affect the mood, but can also adversely affect therapy. The responses to cannabinoid medications depend on the patient's endocannabinoid system activity, the proportion of phytocannabinoids, the terpenoid composition, and the dose used. There is some evidence for a therapeutic use of phytocannabinoids in psychiatric conditions. THC and CBD may have opposing effects on anxiety. Current guidelines recommend caution in using THC in patients with anxiety or mood disorders. In a small number of clinical trials, cannabinoids used to treat cancer, HIV, multiple sclerosis, hepatitis C, Crohn's disease, and chronic neuropathic pain report decreases in anxiety or depression symptoms and presented sedative and anxiolytic effects. Several studies have investigated the influence of potential genetic factors on psychosis and schizophrenia development after cannabis use. THC may increase the risk of psychosis, especially in young patients with an immature central nervous system. There is limited evidence from clinical trials that cannabinoids are effective therapy for sleep disorders associated with concomitant conditions. There is evidence for a possible role of cannabis as a substitute for alcohol and drugs, also in the context of the risks of opioid use (e.g., opioid-related mortality). In this narrative review of the recent evidence, we discuss the prospects of using the psychoactive effects of cannabinoids in treating mental and psychiatric disorders. However, this evidence is weak for some clinical conditions and well-designed randomized controlled trials are currently lacking. Furthermore, some disorders may be worsened by cannabis use.

Keywords: cannabis, cannabinoids, psychiatric disorders, anxiety, depression, dementia, sleep disorders, substitution therapy

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NEUROMODULATING ROLE OF THE ENDOCANNABINOID SYSTEM

Cannabis affects the nervous system in four main domains:

1. Mood (euphoria, unfounded laughter; paranoid or anxious reactions at high doses),
2. Perception (disturbance of the perception of time and space),
3. Somatic symptoms (fatigue, problems with motor coordination, dizziness),
4. Cognitive impairment (confusion, impaired concentration, impaired short-term, and working memory) (1).

Over 110 cannabinoid receptors' ligands have been isolated from *Cannabis sativa*, of which some have neuromodulating properties (2). In the Nineteenth and twentieth centuries, hemp was used to treat sleep disorders, pain, and increase appetite (3). Since the 1990s, after the discovery of the endocannabinoid system (ECS) (4), many publications explaining the mechanism of its action appeared. CB_1 receptors can be found mainly in the central and peripheral nervous systems (5, 6). When discussing the effects of cannabis on the CNS, one should distinguish the effects of the two main cannabinoids, D9-tetrahydrocannabinol (THC) and cannabidiol (CBD), with only THC and its metabolites having a psychoactive effect (2).

Increasing evidence suggests an essential role of the endocannabinoid system (ECS) in the regulation of cognitive abilities, mood, stress, and sleep (7–9). In animal models, pharmacological or genetic disruption of endocannabinoid signaling results in a neurobehavioral response that imitates the classic stress response. It is manifested by activation of the hypothalamic-pituitary-adrenal axis (HPA), increased anxiety, excessive vigilance, agitation, inhibition of feeding behavior, decreased response to rewarding stimuli, and impaired cognitive flexibility (9). Regulation of the stress-response mechanism in short-term stress causes ECS inhibition, while long-term stress stimulates ECS, which alleviates the negative effects of the stressful situation (8). Endocannabinoids (via CB_1 receptors) modulate the functions of all hypothalamic-pituitary-gland axes. Chronic stress seems to reduce the ECS's ability to buffer stress and may induce psychopathology, including anxiety and depression (8). The ECS signaling modulates HPA axis activity in stressful conditions, which may promote psychiatric disorders (10).

The CB_1 receptor plays an inhibitory role in the release of excitatory amino acids and GABA which consequently regulate the release of other transmitters: acetylcholine, dopamine, histamine, serotonin, noradrenaline, prostanoids, and opioid peptides (11, 12). CB_1 receptors are present at very high levels on inhibitory (GABAergic) interneurons and at a lesser extent on excitatory (glutamatergic) terminals (13), as well as on neurons expressing dopamine D_1 receptors, playing a specific role in the repertoire of different emotional behaviors, including social and cognitive activity, which are affected in psychiatric disorders (14–17).

By activating the CB_1 cannabinoid receptor, THC may induce euphoria, cognitive impairment, and intensify negative emotional states, including anxiety. In cell research, CBD has been shown to reduce the CB_1 cannabinoid receptor's activation but is not an antagonist, as it induces intracellular sequelae. CBD may function as a negative allosteric modulator (NAM) of the CB_1 receptor and binds to it at a completely different site than the target THC binding site (18). In the biochemical studies, cannabidiol enhanced endogenous anandamide signaling indirectly through inhibition of the intracellular degradation of anandamide catalyzed by the enzyme fatty acid amide hydrolase (FAAH) (19, 20). The inhibition of FAAH may not be relevant for humans but CBD may inhibit the transport of anandamide (AEA), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) to FAAH by blocking fatty acid-binding proteins (FABPs), which are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (21).

The recent findings suggest that possibly relevant mechanisms of CBD encompass the facilitation of serotonergic neurotransmission via allosteric 5-HT_1A receptor modulation, modulation of glucose homeostasis and inflammatory processes by PPARγ activation, and the interaction with the transient receptor potential vanilloid-1 receptor (TRPV1) (22, 23). Recent evidence suggests the therapeutic potential of cannabinoid-based drugs for a wide range of medical conditions, including neurological and psychiatric disorders (24).

POSSIBLE CLINICAL INDICATIONS OF CANNABIS AND ITS DERIVATIVES

Mood Disorders

The psychoactive effects of cannabis are described as euphoric, calming, anxiolytic, and sleep-inducing. Some of them positively affect mood. On the other hand, in some persons, adverse effects, such as paranoia, irritation, dysphoria, depression, depersonalization, and demotivation, appear (25, 26) (**Supplementary Table 1**). The reactions may depend on the patient's ECS activity, the proportion of phytocannabinoids, the terpenoid composition, and the dose used (bell effect, stimulating effect at a low dose, and inhibitory effect at a high dose) (27). The interaction between these effects can be complex, and therefore requires selecting the appropriate variety and dose by an experienced professional. Noteworthy, the balance of positive and negative effects can change in the same patient during observation and treatment (27). A patient experiencing a mood disorder may not be objective in assessing his or her condition and cannot decide on his or her own to modify treatment. Therefore, professional care and control are essential.

The mood-elevating properties of cannabinoids have been known for a long time and are considered non-toxic. Many patients who do not respond appropriately to standard pharmacological treatments of depression may benefit from medical cannabis use. Cannabinoids may have therapeutic potential in both depression and bipolar disorder (28). The duality of bipolar disorder makes it challenging to treat. Standard pharmacotherapy does not always help with all symptoms and stabilizes both manic and depressive episodes. Some patients successfully add cannabis to ongoing pharmaceuticals, enhancing

their effects, or reducing the side effects of therapy (28, 29). There are reports that cannabis can be a mood stabilizer in bipolar disorder and an adjuvant to lithium therapy (it allows for dose reduction) (28). Nevertheless, there is limited clinical evidence suggesting that cannabis use may cause the onset and worsen the clinical course in bipolar disorder (30, 31).

Early reports in Western medicine describe its effects as "mental joy." Today, many patients admit to using marijuana to relieve symptoms of depression. In an Australian study, 56% of medical cannabinoid users surveyed used them for depression (32).

In U.S. states which legalized marijuana, suicide rates among men aged 20–39 have decreased compared to states where marijuana is illegal (33).

Often depression is secondary to a life-limiting illness. Clinical observations indicate that cannabinoids may provide new treatment options for anxiety or depression secondary to certain chronic diseases. In a study in HIV-infected patients, 86% reported an improvement in depression and 93% in anxiety (34).

In some observational studies of limited quality, cannabis containing CBD and THC in equal proportions attenuated some mood disorders reported in patients using THC-predominant cannabis. An observational study was conducted on 100 patients who used cannabis for multiple sclerosis, chronic pain, nausea, cancer, or psychological problems (35). Patients who used cannabis with low concentrations of cannabinoids (6% THC and 7.5% CBD) experienced significantly less anxiety, dejection, sadness, or depression. They also reported less appetite stimulation compared to those who reported using strains rich in THC (19% THC, <1% CBD) or with medium THC concentration (12% THC, <1% CBD (35). In another observational study, 75 patients suffering from depression, stress, and burnout syndrome were successfully treated with dronabinol, either alone or in combination with other antidepressants. Dronabinol appeared to be a successful antidepressant in a general medicine practice, alone or in combination with other antidepressants (36).

Evidence from a small number of clinical trials of prescribed THC-containing cannabis that patients using cannabinoids to treat cancer, HIV, MS, hepatitis C, Crohn's disease, or chronic neuropathic pain report relief in symptoms of anxiety or depression (9, 35, 37–39).

Most of the psychoactive effects of medical marijuana, such as euphoria, do not occur in every patient. Moreover, less frequently, anti-euphoric, or dysphoric reactions are also observed (40). Patients taking marijuana may experience different effects depending on their current mood, treatment expectations, drug mix, and dosages. If taken at the "wrong time" or during the decreased mood, it can provoke negative thoughts (27). It is essential in adolescents in whom there is a greater risk of depression, other mental disorders, and suicide later in life (41). This can be explained by the immaturity of the central nervous system and neural connections. Still, it is difficult to say whether cannabinoids caused depression or were used in response to depression.

Evidence from pre-clinical and clinical studies indicates a vital role for the ECS in anxiety and mood disorders. The decreased endocannabinoid signaling may entail a depressive-like phenotype. Therefore, boosting of endocannabinoid signaling may appear a novel therapeutic option for the treatment of depression (42). Low doses of CB_1 receptor agonists reduced anxiety behavior and increased antidepressant-like responses in animals (43). Moreover, similar to typical antidepressants, CB_1 receptor agonists seem to increase the central transmission of neurotransmitters (serotonin, noradrenaline) (44, 45). In support of this theory, rimonabant (CB_1 receptor antagonist), approved to treat obesity, was withdrawn after reports of mood and sleep disorders in persons who used it. Patients became more irritable and agitated, and an increase in the incidence of depression and even suicide were noted (46, 47). Despite the psychiatric side effects of rimonabant, there is still interest in the development of CB_1 antagonism as a pharmacological tool for the treatment of metabolic disorders, with a better safety profile. In this context the peripheral CB_1 blockade seems to be a promising therapeutic target (48).

Thus, CB_1 receptors are an important new target in the development of antidepressants. However, the challenge of discovering new cannabinoid antidepressants is to develop CB_1 agonists with selective antidepressant properties, which would reduce adverse psychotropic effects of cannabis use to the smallest possible degree (44).

Anxiety

Cannabis rich in THC induces anxiety behavior. The effect seems to be dose-dependent, with low doses having potentially anxiolytic properties and high doses being ineffective or even increasing anxiety levels (49). When taken in high doses by cannabis-naïve users, THC can cause intense fear and anxiety up to a panic attack. In contrast, long-term cannabis users report reduced anxiety, increased relaxation, and relief from tension (50).

CBD's anxiolytic effects have been assessed in animal models of generalized anxiety disorder, social phobia, panic disorder, obsessive-compulsive syndrome, and post-traumatic stress disorder (PTSD) and in humans (51–53). CBD use's positive anxiolytic effects have been observed in people with generalized social anxiety disorder (SAD) and effectively treat other anxiety disorders and reduce anxiety symptoms (54, 55). The results suggest that CBD reduces anxiety in patients with SAD and this is related to its influence on activity in limbic and paralimbic areas of the brain (56). The anxiolytic properties of CBD have been confirmed in humans and follows the same pattern of an inverted U-shaped dose-effect curve observed in many animal studies. It is necessary to determine the optimal therapeutic doses of CBD for introduction / implementation into clinical practice (57).

Cannabinoids have a sedative and anxiolytic effect and may be assessed by some patients as better than traditional drugs because they do not dull cognitive processes. Still, a significant proportion of patients have the opposite impression and report mental confusion after their use (27). It is commonly believed that a "laid-back" state is felt after cannabis use, but also anxiety

can be exacerbated, even up to a panic attack (58). This problem is troublesome for inexperienced patients without prior training in using the cannabinoids, especially with high THC levels or too high an initial dose (59).

Sleep Disorders

Cannabis and THC have a dose-dependent effect on sleep, with low doses to reduce sleep onset latency and increase slow-wave sleep and total sleep time; and high doses to cause sleep disturbances (60–63).

There is limited evidence from clinical trials that cannabis or THC improve sleep in patients with sleep disorders associated with comorbidities (obstructive sleep apnea syndrome, fibromyalgia, chronic pain, and multiple sclerosis) (64–66). Numerous studies on managing chronic pain, fibromyalgia, and multiple sclerosis report improved sleep in patients, though (67, 68).

Few reports suggest CBD improve REM sleep disturbances and excessive daytime sleepiness (55, 69).

Schizophrenia and Psychosis

Significant evidence from epidemiological, pre-clinical, and clinical studies supports the association between THC (and THC-rich cannabis) and an increased risk of psychosis and schizophrenia (70, 71). However, it seems unlikely that they contribute to the development of mental illness (72). In contrast, based on a genetic approach, cannabis use was associated with an increased risk of schizophrenia than in non-users (73, 74). THC has a pro-psychotic effect, while CBD reduces the occurrence of such disorders (2, 19, 75). However, interactions between THC and CBD may be clinically significant (76).

THC might affect schizophrenia patients differentially causing transient exacerbation of psychotic and cognitive deficits in comparison to control subjects (77). THC-rich varieties may increase the risk of psychosis–especially in young patients whose brains are still developing (74). Novel cannabis strains contain far more THC than old strains, where for centuries, the ratio of THC to CBD has been comparable (2). The cannabis use by adolescents may change the endocannabinoid signaling and pose a potential environmental risk to develop psychosis. In pre-clinical and clinical studies, a potential role of the ECS both in pathophysiology of schizophrenia and as potential therapeutic target, has been found (78).

The evidence does not support or disapprove of CBD as an effective drug for schizophrenia or schizophrenic psychosis. However, emerging evidence suggests CBD's attenuating effect on THC-induced psychosis (19, 76, 79, 80). In a recently published randomized clinical trial of cannabidiol vs. placebo for cannabis use disorder, cannabidiol 400 and 800 mg doses appeared well-tolerated and effective at reducing cannabis use (81). In another randomized clinical trial, an antipsychotic effect of lower doses as an addo-on the multiple antipsychotics in chronically ill patients was not found. However, CBD appeared well-tolerated with no worsening of mood, suicidality, and movement side effects (82).

In several studies, the influence of potential genetic factors on psychosis and schizophrenia development, specifically the interaction function with cannabis use, have been investigated. In adolescence and early adulthood, exposure to various stimuli, including cannabis, can impair the ordinary course of neurobiological development and induce the early onset of schizophrenia in people with a genetic pre-disposition (83, 84). In human peripheral blood mononuclear cells (PBMCs) of schizophrenic subjects, selective alterations of DNA methylation at the promoter of the gene coding for the type-1 cannabinoid receptor (CNR1) were observed and confirmed in a well-validated animal model of schizophrenia, induced by prenatal methylazoxymethanol acetate (MAM). The degree of CNR1 DNA methylation in PBMCs may appear a potential biomarker for schizophrenia (85). In the neurodevelopmental MAM model of schizophrenia, a specific alteration of CB_1 receptors in the pre-frontal cortex, fully reversed by cannabidiol treatment, was found, which may appear a novel potential antipsychotic (86). Likewise, a specific alteration of dopamine D_3 receptors in the MAM model of schizophrenia was found, which seems to be a target of cannabidiol treatment (87). In the THC model of psychopathology there was a specific alteration of CB_1 and D_2 receptors in the pre-frontal cortex of rats, similarly to schizophrenic subjects, which was fully reversed by cannabidiol treatment, as novel potential antipsychotic (88). It is crucial that studies biologically quantify cannabinoid exposure, besides the self-reported use, when investigating its impact on cannabis-related mental health issues (i.e., psychosis, mood disorders, addiction) (89).

Cognitive Disorders and Dementia

Cannabis is associated with cognitive impairment, including short-term memory, attention, executive functions, and psychomotor reaction, and this effect seems residual in heavy users (90, 91). On the other hand, in pre-clinical studies, the ECS demonstrated a protective effect against excitotoxicity, oxidative stress, and inflammation associated with the development of Alzheimer's disease (AD) (92). Animal studies have shown that ultralow doses of THC (0.002 mg/kg) slow down the formation of plaque and tangles and reduce the inflammation caused by their presence, thus supporting the treatment of dementia (93). In post-mortem brain tissue of AD patients and in experimental models of AD, a decrease in neuronal cannabinoid CB_1 receptors, an increase in glial cannabinoid CB_2 receptors, and over-expression of free acid amide hydrolase in astrocytes hint its potential role in inflammatory processes and in neuroprotection (94). Early pharmacological enhancement of brain endocannabinoid levels might protect against beta-amyloid neurotoxicity and its consequences (95). Moreover, beta-amyloid fragments induce a dose-dependent memory deficit, and this effect may be associated with cannabinoid CB_1 receptors in the brain (96).

The clinical evidence for cannabinoids for the treatment of AD is scarce. In a Cochrane (2011) systematic review, the evidence did not support their effectiveness at improving disturbed

behavior or other symptoms in dementia (97). However, only one small-size study met the inclusion criteria Volicer et al. (98). In a newer RCT, with 50 patients enrolled, low-dose THC did not significantly reduce dementia-related neuropsychiatric symptoms, though it was well-tolerated (99).

Opioid Withdrawal Symptoms and Drug Substitution

The first report on the role of marijuana in treating substance abuse (including opiates) was published in The Lancet in 1889 (100).

There is growing evidence to support medical cannabis as an adjuvant or substitute for prescription opioids to treat chronic pain. Cannabinoids combined with opioid analgesics bring on hyper-additive pain relief, which results in a reduction in opioid use and opioid-induced adverse effects (101). Besides that, cannabinoids may prevent the development of opioid tolerance and withdrawal and may even resume the analgesic effect of opioids when the previous dose has become ineffective (101).

Studies show that the use of cannabinoids may be both safe and effective, also in elderly patients, and reduce the number of prescription drugs they receive, including opioids (25).

In a study conducted in Philadelphia, 91 opiate-addicted patients received methadone substitution therapy (102). Patients who used marijuana before the treatment needed less methadone. Additionally, using cannabis during methadone therapy resulted in less expressed withdrawal symptoms (assessed according to the Clinical Opiate Withdrawal Scale, COWS). The consumption of marijuana in the initial phase of substitution therapy, when strongly expressed withdrawal symptoms were present, was higher than in the subsequent ones when withdrawal symptoms retreated (102). Another randomized clinical trial revealed the potential of CBD to reduce cue-induced craving and anxiety as a treatment option for opioid use disorder (103).

The effectiveness of cannabinoids in alleviating withdrawal symptoms associated with opioid abstinence or dose reduction of opioid analgesics can be explained by overlapping neuroanatomical distribution, convergent neurochemical mechanisms, and comparable functional neurobiological properties of ECS and the opioid system (104, 105).

Medical cannabis's benefits were assessed in Canada on 404 patients in an anonymous survey that subjectively assessed the effects of medical cannabis on the use of alcohol and illicit psychoactive substances (106). Cannabinoids reduced withdrawal symptoms and resulted in less frequent side effects and better control of symptoms of existing diseases than other pharmaceuticals.

There is a growing consideration in substituting alcohol, opioids, and other psychoactive substances with cannabis to reduce abstinence-associated withdrawal symptoms and the risks of their use (e.g., opioid-related mortality). Pre-clinical studies suggest that some cannabinoids (such as THC) may ease opioid withdrawal (107, 108). In an observational study, cannabis use alleviated opioid withdrawal symptoms, but the clinical evidence is insufficient to draw any conclusive recommendation (109, 110).

Nevertheless, it is essential to reiterate that long-term cannabis use can cause a motivational syndrome and addiction (111).

TABLE 1 | The neuropsychiatric effects of tetrahydrocannabinol (THC) and cannabidiol (CBD) (2).

THC	CBD
• Psychoactive (euphoria or dysphoria, anxiety in some new users)	• No psychoactive activity • Counteracts psychotropic effects of THC (short-term memory and cognitive disorders) • Possible anti-psychotic effect
• Relaxation and bliss	
• Analgesic	• Analgesic • Anxiolytic and antidepressant
• Soporific (secondary effect, dose-dependent)	• Induces sleep, suppresses waking-up, regulates sleep disorders (also anxiety related)
• Stimulates appetite	• Suppresses appetite

CONCLUSION

The neuroprotective role of the endocannabinoid system is still the subject of extensive research. Preclinical research suggests its modulatory effect on numerous neurological, emotional, and psychiatric symptoms. As discussed in this article, there is weak evidence for cannabinoids' beneficial results in anxiety, mood, and sleep disorders, as summarized in **Table 1**. There is also a growing interest in cannabis use as a substitute for psychoactive substances. On the other hand, several studies report the development of psychosis and cognitive impairment. The evidence supporting cannabis use in psychiatric disorders is insufficient and of low quality yet. Further translational research is necessary to understand the pharmacodynamics in humans, and clinical studies are required to assess the risks and benefits of cannabis use.

AUTHOR'S NOTE

There is an increasing interest in cannabis use in neuropsychiatry. The evidence is relatively scarce or of low quality or comes from the pre-clinical research. Nevertheless, it supports the endocannabinoid system's role in regulating stress, mood, cognitive abilities, and sleep. The effects such as euphoric, anxiolytic, calming, or sleep-inducing are well known from recreational use experiences. There is increasing evidence for cannabinoids in the therapeutic use for anxiety, depression, insomnia, psychoses, and opioid substitution. In this mini-review, we tried to signal the research's key directions on cannabis in psychiatry. Where possible, we presented the clinical evidence to provide an overview of the state of knowledge. We avoided too detailed explanation of the pathophysiology or etiology, bearing in mind that the special issue "The Endocannabinoid System: Filling the Translational Gap between

Neuroscience and Psychiatry" will consist of translational research articles and problem-specific papers. We hope that the article will be a kind of roadmap of the prospects of current and research.

AUTHOR CONTRIBUTIONS

MG: review plan. MG, MŁ, and TD: data collection, interpretation, and proof reading. MG and TD: writing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.620073/full#supplementary-material

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Endocannabinoid Gene × Gene Interaction Association to Alcohol Use Disorder in Two Adolescent Cohorts

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Genetic markers of the endocannabinoid system have been linked to a variety of addiction-related behaviors that extend beyond cannabis use. In the current study we investigate the relationship between endocannabinoid (eCB) genetic markers and alcohol use disorder (AUD) in European adolescents (14–18 years old) followed in the IMAGEN study ($n = 2,051$) and explore replication in a cohort of North American adolescents from Canadian Saguenay Youth Study (SYS) ($n = 772$). Case-control status is represented by a score of more than 7 on the Alcohol Use Disorder Identification Test (AUDIT). First a set-based test method was used to examine if a relationship between the eCB system and AUDIT case/control status exists at the gene level. Using only SNPs that

are both independent and significantly associated to case-control status, we perform Fisher's exact test to determine SNP level odds ratios in relation to case-control status and then perform logistic regressions as *post-hoc* analysis, while considering various covariates. Generalized multifactor dimensionality reduction (GMDR) was used to analyze the most robust SNP × SNP interaction of the five eCB genes with positive AUDIT screen. While no gene-sets were significantly associated to AUDIT scores after correction for multiple tests, in the case/control analysis, 7 SNPs were significantly associated with AUDIT scores of > 7 ($p < 0.05$; OR<1). Two SNPs remain significant after correction by false discovery rate (FDR): rs9343525 in *CNR1* ($p_{corrected}$ =0.042, OR = 0.73) and rs507961 in *MGLL* ($p_{corrected}$ = 0.043, OR = 0.78). Logistic regression showed that both rs9353525 (*CNR1*) and rs507961 (*MGLL*) remained significantly associated with positive AUDIT screens ($p < 0.01$; OR < 1) after correction for multiple covariables and interaction of covariable x SNP. This result was not replicated in the SYS cohort. The GMDR model revealed a significant three-SNP interaction ($p = 0.006$) involving rs484061 (*MGLL*), rs4963307 (*DAGLA*), and rs7766029 (*CNR1*) predicted case-control status, after correcting for multiple covariables in the IMAGEN sample. A binomial logistic regression of the combination of these three SNPs by phenotype in the SYS cohort showed a result in the same direction as seen in the IMAGEN cohort (BETA = 0.501, $p = 0.06$). While preliminary, the present study suggests that the eCB system may play a role in the development of AUD in adolescents.

Keywords: alcohol use disorder, cannabinoid receptor 1, CNR1, DAGL, endocannabinoid system, MGLL

INTRODUCTION

Substance use disorders are a growing concern across the world, with an estimated 31 million users worldwide suffering from drug use disorders. After alcohol and tobacco, cannabis ranks as the most used drug worldwide (1). Moreover, those who use cannabis are more than five times more likely to have an alcohol use disorder (AUD) (2). Considering that the endocannabinoid (eCB) system is responsible for the physiological consequence and subjective "high" of cannabis, much attention has been paid to the eCB role in the development of various substance use disorders. Cannabinoid receptors and related enzymes are expressed in many of the reward centers of the brain: nucleus accumbens (NAc), ventral tegmental area (VTA), amygdala, and basal nucleus of the stria terminalis (BNST) (3, 4). These eCB levels are affected by ethanol (5), and the eCB system plays a role in the development of AUD and other substance use disorders in humans (4). Basavarajappa and colleagues (6) demonstrated that acute ethanol use has been associated with an increase in eCB signaling, while others have reported that alcohol use decreases eCB signaling (7, 8). Moreover, as is the case with other drugs of abuse, eCBs mediate the reward signals associated with alcohol use (9). Overall, the underlying evidence shows that the eCB system is modulated by ethanol use, and this same system may play an independent role in AUD (10).

The first eCB receptor isolated, of which tetrahydrocannabinol (THC) is also a ligand, is the cannabinoid receptor one (CB1) (11, 12). Binding to this receptor and a second cannabinoid receptor (CB2) are the two main eCB agonists, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These agonists—which are not stored in vesicles—are produced through an enzymatic cascade in a Ca^{22++} dependent manner, and then are rapidly degraded by specific enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) plays a crucial role in the synthesis of AEA, which then binds to CB1. 2-AG is synthesized by diaglycerol lipase (DAGL).

It has been shown that polymorphisms in the *CNR1* gene, the gene coding for the CB1 receptor protein, are associated with a range of diseases, psychiatric disorders, and substance use (13–15). Many studies have assessed the various aspects of the eCB genes and their relationship with substance use disorders and risk-taking behavior. The single nucleotide polymorphism (SNP) rs1049353 in the *CNR1* gene has been associated with severe alcoholism (minor A allele) (16), heroin addiction (major G allele) (17), and impulsivity (18). Furthermore, haplotype blocks within the *CNR1* gene have been associated with addiction and addictive behavior (19, 20). Polymorphisms in the *FAAH* gene have also been associated with problem drug use and addiction (21, 22). In contrast, there have been relatively few studies examining the *MGLL* gene, the gene coding for the MAGL enzyme, and the *DAGL* in association with drug dependence (23–25). Among these, only one study has found a positive association between SNPs of the *MGLL* gene and drug dependence (25), while no studies have reported a significant association between *DAGL* and any form of drug abuse. Moreover, many of the original findings reporting an association between SNPs located in genes of the eCB and various drug abuse behaviors have not

been replicated (4, 26), suggesting the possibility of false positive results in these candidate gene approaches. Nevertheless, while there are conflicting results among studies, the candidate gene literature suggests that genes related eCB proteins may play a role in the development of substance use problems.

While candidate gene findings in psychiatric genetics have been widely criticized for replication failure, particularly with respect to GWAS and meta-analysis (27), candidate gene approaches in addiction research have identified genetic markers that have been confirmed in GWAS and meta-analysis (28). This is perhaps related to particularly heritable nature of addictive behaviors compared to other psychiatric conditions, or to the fact that candidate gene approaches can be directly informed by pharmacogenetic studies on how drugs of abuse interact with the brain's neurochemistry. Others have argued (29) that the failure to replicate candidate gene findings through GWAS and meta-analysis does not necessarily suggest the findings are false. The candidate gene findings may represent particular endophenotypes of sub-populations, which may account for a portion, albeit small, of genetic influence on the phenotype in question. Thus, other groups have utilized novel methodologies, such as gene-set approaches, to analyze hypothesis-based questions in psychiatric genetics and addiction medicine. Recently, one group, utilizing said gene-set approaches, found that *MGLL* and the SNP rs604300 interact with childhood sexual abuse to predict cannabis dependence symptoms (25). Considering our relatively limited understanding of the roles of the various endocannabinoid genes in the pathogenesis of addictive behaviors, and the lack of robust findings at the individual SNP, or GWAS levels, gene-set, and system-based approaches remain of interest (25, 30). Thus, the current study employs a gene-set based approach in an attempt to shed light on the role of the eCB system in the pathogenesis of addictive behaviors.

Given the effect of alcohol on the eCB system (5) and the purported relationship between eCB SNPs and the risk for substance use disorder, we assessed the association between eCB genetics and alcohol abuse behaviors in the IMAGEN cohort (31). The IMAGEN cohort is a European cohort of 2,087 adolescents recruited in France, UK, Ireland, and Germany. Endocannabinoid genetic influence was studied through a candidate gene approach. Multiple SNPs in eCB genes that have been previously examined (*CNR1, FAAH, MAGL, DAGLA*) as well as genes that have not yet been investigated (*NAPEPLD*) were analyzed in the context of alcohol use disorder (AUD). To understand this relationship, a three-tiered approach was used. First, a set-based test (32) is utilized to study, at the gene level, the link between the eCB system and alcohol abuse behavior. Through this approach we also identify SNPs that are significantly and independently associated to positive AUD screening, and these SNPs are selected for further study using a case/control analysis and subsequent logistic regression. Finally, while some studies have investigated the interaction between two eCB genes and addictive behavior (33, 34), none have examined the eCB system as a whole. Considering the complex interplay between the multiple eCB ligands (AEA and 2-AG among others) and various receptors (CB1, CB2, etc.) in their relationship to addictions (4), we hypothesize that a single genetic marker association study could not account adequately for the multifaceted role the eCB system plays in risk for AUD. A new wave of candidate gene studies have explored more complex gene-gene interactions, using various methods of multifactor dimensionality reductions analyses to yield promising results such as predicting outcomes in breast cancer treatment (35), in determining genetic biomarkers to predict antidepressant response (36), and further understanding the genetic influences of nicotine addiction (37). Here, we utilized Generalized Multifactor Dimensionality Reduction (GMDR) to understand the effects that the multiple eCB genes may have on each other and their combined influence on alcoholic behavior in adolescence. To replicate the results, genetic and alcohol use data were used from the Saguenay Youth Study (SYS), a two-generational study comprised of 1,029 French-Canadian adolescents and their parents.

MATERIALS AND METHODS

Participants

The IMAGEN study is a longitudinal imaging genetics study of 2,087 healthy adolescents, mostly of European descent. Detailed descriptions of this study, genotyping procedures, and data collection have previously been published (31). The IMAGEN cohort has been repeatedly assessed on substance use outcomes at 14, 16, and 18 years of age. The multicentric IMAGEN project had obtained ethical approval by the local ethics committees (at their respective sites) and written informed consent from all participants and their legal guardians. The parents and adolescents provided written informed consent and assent, respectively. All datasets were de-identified by using codes for individuals. See Schumann et al. (31) for a more detailed description of the IMAGEN cohort.

The current study used data for all 2,087 individuals who completed the IMAGEN assessment battery at 14, 16, and 18 years of age and who contributed their genetic data at 14 years of age. Of those followed at 16 or 18 years of age, three individuals had unassigned sex according to sex determination analysis in PLINK1.9 (38) and were thus excluded from the genetic analyses. Moreover, 11 individuals did not answer the Alcohol Use Disorder Identification Test (AUDIT) at any time point and were thus removed from the genetic analyses. Eleven pairs of siblings were a part of the IMAGEN database, and thus one sibling from each pair was removed from the study, according to the methods published (39). European ancestry was determined using Admixture (40) using HapMap III (41) as a reference population. Eleven individuals with non-European ancestry were removed prior to analysis. Thus, in this study there was a total of 1,043 female and 1,008 males. A summary of the individuals can be seen in **Table 1** and **Supplementary Table 1**.

Phenotype Evaluated

Alcohol Misuse

AUDIT is a self-report questionnaire developed by the World Health Organization and validated (42) to screen for heavy drinking and current alcohol dependence. Individuals were

TABLE 1 | Description of subjects in IMAGEN and SYS.

Cohort		IMAGEN	SYS
N (female %)		2051 (50.8%)	772 (52.07%)
N family		2051	401
Age (SD)		14 to 18 years[c]	15 years (1.85)
AUDIT[a]	Control	1476	-
	Case	575	-
GRIP[b]	Control	-	724
	Case	-	48

[a] *IMAGEN subjects are classified by status with AUDIT score, case is > or = to 8 and control < 8;* [b] *SYS subjects are classified by status with GRIP score, case > or = to 2 and control < 2.* [c] *IMAGEN cohort is a longitudinal cohort, so it's not possible to calculate the standard deviation (SD).*

considered to screen positive for risk for AUD and were included in the case group if they scored 8 or more on the AUDIT (case-control status). While other studies focusing on adolescent alcohol abuse used a less stringent cut-off (43–45), the more stringent cut-off of 8 was chosen as this is the cut-off with the strongest sensitivity and a favorable specificity across all studies (46). Four AUD scores were derived: "Any AUD" representing having screened positive for AUD at any timepoint from 14–18 years of age, and then individual dichotomized scores for each of the time points, 14, 16, and 18 years. For details about choice of cut-off, see **Supplementary Methods**.

Covariates

Covariables include sex, the first six genetic principal components, parental alcohol abuse, and parental education. Parental education was taken from the parent-report questionnaire using the educational categories specified in the European School Survey Project on Alcohol and Other Drugs (ESPAD+) questionnaire. Risk for AUD in parents was measured using the AUDIT obtained at the first two time points in IMAGEN. If ESPAD+ and AUDIT information were missing at the 18-year-old time point, the most complete and recent information was used at this time point. If a parent had signaled a positive AUDIT at any time, they were flagged as such. Moreover, if parental information was missing, individuals were not included in the logistic regression.

Pipeline of SNP Selection

The genotyping was run using the Illumina Quad 610 chip and 660Wq at the "Centre National de Genotypage" (Paris, France). Only autosomal SNPs were kept for this study. SNPs with a minor allele frequency (MAF) of <5%, a missing SNP rate of 10%, or SNPs that did not respect Hardy Weinberg Equilibrium (HWE) ($<1 \times 10^{-6}$) were also removed from this study. All available SNPs in the genes of interest (*CNR1, NAPE, FAAH, MGLL, DAGLA*) within ±10 kb (to include promoter and flanker regions) were then selected. Gene length and location were obtained using the UCSC Genome Browser. The SNP coordinates were updated from hg18 to hg19 using Illumina information and the liftover tool from the genome browser (http://genome.ucsc.edu/cgi-bin/hgLiftOver). Nevertheless, SNP information was scarce on the *CNR2*, and as such, the gene was not included in this study. A summary of the locations and details of each SNP (gene, chromosome, base pair, function, etc.) can be seen in **Supplementary Table 2**.

Statistical Analysis

Sixty-nine SNPs appearing across five cannabinoid-related genes were analyzed for their relation to problematic alcohol consumption. As a primary analysis, we first conduct three set-based tests using parameters of varying stringencies, to study the relationship between 5 endocannabinoid gene-sets (*CNR1, NAPEPLE, FAAH, MGLL, DAGLA*). The parameters that were adjusted between the tests were *p*-value for significant variants between tests, r^2 of variant pairs, and maximum set size. Data in all three set-based tests underwent 10,000 label-swapped permutation as well, using the—perm function in PLINK1.9. The first test was the default test in PLINK1.9, with a *p*-value of 0.05, r^2 of 0.5, and a set-max of 5; the second test had a *p*-value of 0.05, r^2 of 0.3, and set-max of 3; while test 3 had a *p* of 0.01, r^2 of 0.1, and set-max of 2. Tests 2 and 3 were more stringent and were run to challenge the data, to ensure robustness of our results. Statistical significance for set-based test was determined using a Bonferroni corrected empirical *p*-values of $p < 0.01$ (0.05/5 genes). Burden and optimized sequence kernel association tests (SKAT, R package) (47) were used to analyze the joint effects of SNPs (in gene sets). These analyses were performed on three groups of variants: (a) Set 1, (b) Set 2, (c) Set 3 defined with gene set PLINK analyses. We resampled 10,000 times to compute empirical *p*-values (*p*-values were adjusted controlling for family-wise error rate) for the analyses (with "bootstrap" option).

Next, to determine SNP level odds ratios (OR) case-control analysis was run on the SNPs that were nominally significant and independent after set-based analysis, using Fisher's exact test. In the case-control analysis, false discovery rate (FDR) was used to correct for multiple tests. To test the robustness of these findings after controlling for various relevant covariates, a logistic regression was performed that included only the SNPs that remained significant after correction for multiple tests, sex, the first six ancestry components, parental AUDIT flag, and parental education were included in the logistic model. In *post-hoc* analysis, for SNPs that significantly predicted case-control status, after controlling for covariates, we control for potential confounding of interaction (48) and include the interaction of the covariate of no interest by SNP (see **Supplementary Methods** for descriptions of the covariables and **Supplementary Figure 1** for results of principal component analysis). The set-based test, Fisher exact test, and logistic analyses were all carried out using PLINK program (38).

Generalized Multifactor Dimensionality Reduction

In order to test the replicability of these findings across a different analytic strategy, GMDR was employed to analyze the SNP x SNP interaction with phenotype. GMDR (v1.0) is a free open source tool for identification of interactions, developed by Guo-Bo Chen (49). This program was used to screen for the best interaction combinations among the 69 SNPs and the phenotype of interest.

Permutation with 10,000 shuffles providing empirical *p*-values to measure the significance of an identified model was used. For these analyses, logistic regression with the same covariables as described above were performed. For more information on the GMDR method see Lou et al. (49).

SYS Replication Cohort

Genetic and alcohol-use data from the Saguenay Youth Study (SYS) were used to replicate the findings. The SYS is a two-generational study comprised of 1,029 adolescents and 962 parents (50). For descriptive characteristics of the participants included in the replication see **Supplementary Table 3** and **Table 1**. All individuals were genotyped using whole blood samples from which DNA was extracted. The genotyping was performed at "Centre Nationale de Génotypage" for 610Kq (No. arrays = 599) and at the Genome Analysis Centre of Helmholtz Zentrum München (Munich, Germany) for HOE-V12 (No.arrays = 1,395). Genetic information was imputed following previously published methods (50) and after that, the 69 SNPs studied were extracted. Detailed descriptions of the cohort, genotyping, and data collection have previously been published (50, 51).

Participants were recruited over a 10-year period. Once recruited, adolescents provided genetic material and underwent a detailed assessment in several domains. Alcohol-use data for the SYS cohort were obtained *via* a self-report questionnaire developed specifically for the SYS to assess mental health and substance use based on validated protocols (52). The items from this questionnaire that were deemed to overlap sufficiently with AUDIT questions are listed in **Supplementary Table 4**. Of 1,029 adolescents in the SYS cohort, 772 adolescents aged 14 years and older had completed both the SUD assessment and provided genetic information, and were therefore included in this study.

In the replication of the case-control study, we studied the 7 SNPs found in the set-based test. Description of SNPs can be found in **Supplementary Table 5**. Two statistical models were used to study the replication group. To study the native continuous phenotype, a model based on the quasi-poisson distribution was used. The participants were also separated into four different drinking groups, based on scoring distribution. A binomial logistic model was then used separating the participants into controls (groups 0–1; low alcohol use) and cases (groups 2–4; high alcohol use). Both models considered sex, age as covariables and family ID as random effect. Statistical analyses were performed using R, with the glmmTMB library, version 3.5.3 (https://www.R-project.org/).

RESULTS

Set-Based Tests: Identifying Candidate SNP

The three set-based tests were run, with varying results (**Supplementary Table 6**). In the first set-based test, nine SNPS returned with nominal *p*-values of <0.05, of which seven also passed linkage disequilibrium (LD) criterion. Through the first set-test criterion, only the CNR1 gene-set had a significant empirical *p*-value ($p = 0.022$), but this was not significant after correction for multiple tests. Within this set, only rs9353525 was significantly and independently related to dichotomized AUDIT scores. In the second set-based test, the same nine SNPS returned with nominal *p*-values of <0.05, of which five SNPs passed the LD criterion. Nonetheless, no gene sets were significantly associated to case control status ($p > 0.05$). Finally, four SNPs returned with a *p*-value < 0.01 in the third test, with two SNPs passing LD criterion. No genes remained significant after correction for multiple testing ($p_{FDR} > 0.05$). As mentioned above, the seven SNPs that had marginal *p*-values of <0.01 in the first set-based test, and that passed LD criterion ($r^2 < 0.5$), were extracted, and only these were analyzed in the case-control analysis and logistic regression analysis. SKAT demonstrated similar results for the CNR1 gene (**Supplementary Table 7**).

Case-Control Analysis and Sensitivity Analysis

In the case-control analysis of the IMAGEN cohort, which considered cases as individuals who scored eight or more on AUDIT at any time point (ALL), all 7 SNPs analyzed were significant ($p < 0.05$) (**Table 2**). All of the minor alleles were protective against having a case control status (OR < 1). Two SNPs remained significant after correction by FDR: rs9343525 in *CNR1* ($p_{FDR} = 0.043$, OR = 0.73) and rs507961 in *MGLL* ($p_{FDR} = 0.043$, OR = 0.78). A multivariate logistic regression analysis was done for the two SNPs that were significant after FDR correction in the Fisher test (**Table 3**). As a first *post-hoc* analysis logistic models were done for significant SNPs, at each time point (14, 16, and 18), as well as for any positive screen (ALL) for case-control status. After controlling for the effects of the first six principal components, sex, parental AUDIT scores (at any time), and parental education, both rs9353525 and rs507961 were still significantly associated with positive AUDIT screen in the ALL analysis (**Table 3**) ($p < 0.01$), with both SNPs minor allele acting as protective factors (OR < 1). In our *post-hoc* analysis, we find a significant interaction between rs9353525 and PC1 and PC6, as well as a significant interaction of rs507961 and PC3, suggesting that the genetic background, captured by the principal components, may modify the genetic effects of the SNPs on AUDIT scores. For complete results of logistic regression see **Supplementary Table 9**, and see **Supplementary Table 10** for results of *post-hoc* interaction analyses. Finally, we conducted *post hoc* analyses to study the association between AUDIT scores and SNPs of interest at each IMAGEN time point (14, 16, and 18 alone). After correction for multiple testing, none of the *post-hoc* analysis demonstrated significant results (see **Supplementary Results** for detailed results).

In the replication cohort, rs484061 was significantly associated with problematic alcohol use ($p = 7.47^{*}10^{-6}$) in the binomial model. None of the other SNPs in the replication analysis had a significant result, after correction for multiple tests (**Supplementary Table 8**).

GMDR: SNP × SNP Interactions

A GMDR model was used to screen for the most robust interaction of combinations for the 69 SNPs in the candidate genes and case control status. For the one and two-SNP

TABLE 2 | Table of results for case/control analysis ALL.

SNP	A1	A2	Freq AC	Freq AU	OR	Pvalue	FDR Pvalue
rs782446	C	A	0.22	0.26	0.83	0.024	0.081
rs484061	G	A	0.46	0.51	0.83	0.0091	0.055
rs604300	A	G	0.09	0.11	0.77	0.033	0.085
rs507961	T	C	0.20	0.24	0.78	0.0047	0.043
rs9353525	A	G	0.10	0.14	0.73	0.004	0.043
rs4729873	G	A	0.33	0.38	0.85	0.027	0.081
rs10488693	T	C	0.06	0.08	0.73	0.026	0.081

A1, minor allele; A2, major allele; Freq AC, frequency of minor allele in cases; Freq AU, frequency of minor allele in controls; OR, odds ratio. FDR Pvalue, p-value after false discovery rate correction.

TABLE 3 | Table of results for logistic model with AUDIT and rs9353525 and rs507961.

Phenotype	SNP	A1	NMISS	BETA	OR	STAT	*P*
AUDIT ALL	rs507961	T	2030	−0.27	0.76	−3.06	**0.002**
	rs9353525	A	2026	−0.30	0.74	−2.61	**0.009**
AUDIT for 14	rs507961	T	2024	−0.24	0.78	−1.10	0.27
	rs9353525	A	2020	−0.22	0.81	−0.78	0.44
AUDIT for 16	rs507961	T	1535	−0.19	0.83	−1.49	0.14
	rs9353525	A	1532	−0.46	0.63	−2.49	**0.01**
AUDIT for 18	rs507961	T	1243	−0.30	0.74	−2.59	**0.01**
	rs9353525	A	1240	−0.32	0.73	−2.05	**0.04**

A1, minor allele. NMISS, number of non-missing individuals. OR, odds ratio. Stat, coefficient t-statistic. $p < 0.05$.

TABLE 4 | Table of results for the best combinations defined by GMDR for 69 SNPs for AUDIT.

Model	Training accuracy	Testing accuracy	Sign test (*p*)	CV consistency
[rs806368]	0.53	0.49	8 (0.94)	15/20
[rs806368, rs10488693]	0.55	0.50	12 (0.17)	15/20
[rs484061, rs4963307, rs7766029]	0.58	0.541	16 (0.006)	19/20

Model, SNPs included in the model; Sign test, sign test result with p-value in parentheses; CV consistency, cross validation consistency.

models, no significance was found p>0.05. However, we found a significant three-SNP model ($p = 0.006$) involving rs484061 (*MGLL*, intron), rs4963307 (*DAGLA*, intron), and rs7766029 (*CNR1*, downstream-gene) with AUDIT positive screens. An interaction between rs484061, rs4963307, and rs7766029 was significantly associated with case-control status, with a combination of G/A;G/A;C/C or G/G;G/G;C/C conferring protection against problem drinking in the cohort ($p = 0.004$ and $p = 0.02$, respectively; **Supplementary Table 11**). The cross-validation consistency of this three-locus model was 19/20. The testing accuracy of the three SNP model (54%) was greater than the testing accuracy of either the one SNP (49%) or two SNP models (50%) (**Table 4** and **Figure 1**). This result was verified by re-analyzing the model using 10 different random seeds, and this model remained significant for each seed. An analysis of the same three SNP combination in the SYS cohort, binomial logistic model, showed a result in the same direction as seen in the IMAGEN cohort (BETA = 0.50, $p = 0.06$), and the distribution of at risk and protective combinations of SNP with phenotype is comparable to that of the IMAGEN population (**Supplementary Tables 11**, **12**).

DISCUSSION

Although no gene-sets were significantly predictive of binary AUDIT scores, after correction for multiple tests, our case/control analysis suggest that two SNPs, rs507961 (*MGLL*) and rs9343525 (*CNR1*), are associated with problem drinking and remained significantly associated after correction for multiple tests. The SNPs remained significantly associated to case-control status in logistic regression, while considering multiple covariables, and the interaction of these covariables and the SNPs in question. The results of our logistic regression were not replicated in the replication cohort. To our knowledge, one study (25) had investigated rs507961 in *MGLL* in relation to substance use disorders; however, the association did not remain significant after correction for multiple tests. While rs507961 is intronic in *MGLL*, this SNP plays a role in histone regulation of this gene in the brain (**Supplementary Table 13**). The robustness of our result confers evidence that carrying the minor T allele may in fact confer protection against problem drinking. Moreover, no study has investigated the relationship between rs484061, another *MGLL* SNP, and substance use disorders. The recurrence of rs484061 in both the GMDR model and case-control analyses suggests that being a carrier of this SNP protects against risk for AUD. While rs484061 was significantly associated to positive AUDIT screens in the case-control analysis of the IMAGEN cohort ($p = 0.009$, pFDR = 0.055) and replicated in SYS ($p = 7.47^{*}10^{-6}$), it was significantly associated to lower alcohol use. Our results suggest a role for *MGLL* in AUD but work in larger cohorts is needed to confirm this result.

The second SNP that remained significant after correction for multiple tests in our case-control analysis was rs9353525. It is localized in an intergenic region <10 Kb of the 3′ region of *CNR1*. In an attempt to understand the biological role that this SNP plays in the regulation of *CNR1* expression, we scanned the various available databases for potential roles; however, this SNP is relatively understudied. While this SNP was not associated with higher rates of alcohol use in the SYS cohort, this SNP is in strong linkage disequilibrium with rs806368 (at 78% for allele T with G respectively for rs806368 and rs9353525). The rs806368 has been associated to alcohol dependence in other studies (53). We also investigated rs806368 in our cohort, using the same case-control analysis as for our other SNPs, and the major allele is associated with likelihood of

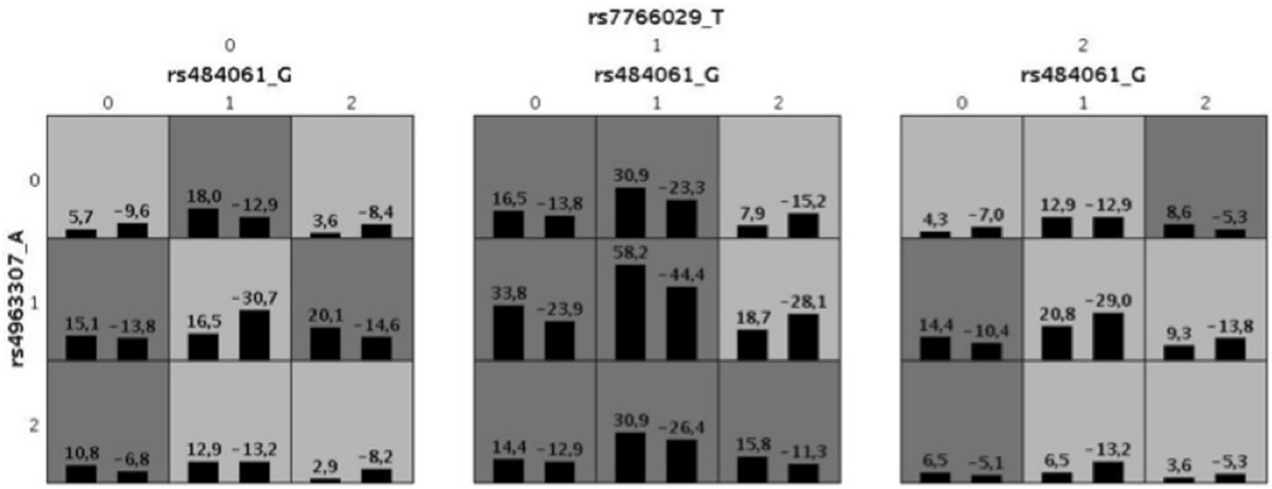

FIGURE 1 | Illustration for the best combination defined by GMDR for 69 SNPs for AUDIT. The allele code is defined by minor allele numbers of rs484061 (allele G), rs4963307(allele A) and rs7766029 (allele T). The numbers above the histogram bar, indicate the sum of "positive" (above the averaged score = 0) and "negative" (below the averaged score = 0) scores by the combination of genotypes. Also, the dark gray indicates a high-risk combination of the genotypes with alcoholism and light gray for low risk. It defined by sum of positive and negative score, when it's < 0 for low-risk and > 0 for high-risk.

reporting a clinically significant AUDIT score at any timepoint in the IMAGEN cohort ($p = 0.007$ OR = 1.28). Moreover, this result remains significant after controlling for the various covariates described above in the IMAGEN cohort ($p = 0.007$; see **Supplementary Tables 14, 15**). Taken together, these results suggest that the haplotype block containing both of the major alleles of rs9353525 and rs806368 plays a role in the development of AUD in adolescents.

A GMDR model was used to screen for the gene x gene interaction that would be most associated to problem drinking, across genes showing a signal in previous analyses. We found a significant interaction involving rs484061 (*MGLL*), rs4963307 (*DAGLA*), and rs7766029 (*CNR1*) that predicted clinically significant AUDIT scores after correction for covariates. Each of these three SNPs are associated to loci, which are key regulators of gene expression (**Supplementary Tables 13, 16**). This observation was supported by the consistency of the result in the GMDR, across IMAGEN and SYS GMDR results ($p = 0.06$) (**Supplementary Table 8**). The similar distribution pattern of problem drinkers within the SYS cohort suggests that the marginal result in the SYS cohort is probably due to a lack of statistical power. The SYS cohort comprises a relatively young sample (mean age = 15 years old), as compared to the IMAGEN cohort, which includes data from individuals when they are 14, 16, and 18 years of age. As such, many of the participants in the SYS cohort have not had their first contact with alcohol, and therefore might not have developed heavy patterns of drinking. This marginal effect should be investigated using data from this sample as it ages, to explore whether the effect becomes larger and more significant when substance use behaviors are assessed during the typical age when substance use disorders have their onset.

Endocannabinoid Interactions in the Brain and Emotional Regulation

The GMDR analysis suggests that a certain combination of SNPs along the *CNR1-DAGLA-MGLL* genes protect against or pose a risk for alcoholism, by presumably modulating DAGLA and or MGLL expression and subsequently 2-AG levels. The DAGLA protein (encoded by *DAGLA*) catalyzes the formation of 2-AG, which then acts as an agonist of CB1. Then, 2-AG is promptly degraded by MAGL (encoded by *MGLL*). Also, 2-AG has been shown to play a key role in the regulation of the hypothalamic-pituitary-adrenal (HPA) stress response axis (54), which is altered in alcohol addiction (55). In response to increased corticosterone, 2-AG levels increase in the medial prefrontal cortex and paraventricular nucleus of the hypothalamus, and act as a negative feedback signal to inhibit the HPA axis and terminate the acute stress response (54). While 2-AG levels increase *in situ*ations of chronic stress, it is theorized to play a role in stress habituation (54). Along the same line, 2-AG has also been shown to play a role in the reduction of stress induced-anxiety in a role mediated through the actions of MAGL and DAGLA (54). MAGL antagonists have been shown to have a strong anxiolytic effect in rodents (56, 57). Knockout studies have shown that *DAGLA (-/-)* mice, which have large reductions in brain 2-AG levels, have increased anxiety-like symptoms (58, 59). Moreover, the anxiety-like state seen in animal models of alcohol dependence and withdrawal symptoms are mediated by corticosterone-releasing factor release in the central nucleus of the amygdala (CeA) (60). A recent study in alcohol dependent rodents found that 2-AG levels were decreased in the CeA of these animal models, and that inhibition of MAGL, increasing 2-AG levels, ameliorated abstinence-related anxiety and excessive alcohol intake (61). Mice exposed to chronic mild stress have reduced levels of *DAGLA* expression, and reduced *DAGLA*

expression in this same study was significantly associated to increased preference for alcohol (62). The study by Ishiguro and colleagues was also the first to link SNPs in the *DAGLA* gene and alcoholism in humans (62). Our study supports the hypothesis that suggests that the eCB system plays a role in the development and/or maintenance of AUD in adolescents. Previous findings suggest that this vulnerability might be achieved by affecting sensitivity to anxiety-like symptoms and influencing reward sensitivity to alcohol intake and warrants further study.

While the results of this study suggest a relationship between eCB genes and AUD, we must acknowledge that the results of this study are preliminary and modest. First, many researchers have called hypothesis-based candidate gene approaches into question (27, 63, 64). This is due to the fact that, while very large GWAS studies consistently report that individual SNPs exert very small effects on complex phenotypes such as addiction, most published studies in the field report significant results, even with relatively small sample sizes (27). Considering that these small candidate gene studies may be underpowered (65, 66) (including ours), the significant results reported in the past are most likely false positive (27). It is also possible that this might be the case in the current study; however, the use of a replication sample provides a context in which to interpret the findings and make conclusions about generalizability of the findings.

Moreover, we were unable to replicate many of the previously reported findings in relation to substance use and eCB genes. This is because our set-based test eliminated many of the previously reported SNPs as they were non-independent according to our criteria. Moreover, some SNPs that are previously reported, mainly rs2023239 (9, 34) and rs6454674 (53, 67, 68), are not assessed in the assay chips used in the present study or were too infrequent in our cohort for analysis. This was also the case for SNPs within *CNR2* that have been previously evaluated for their relationships with substance use. Considering that our findings were most robust within the analysis considering all timepoints, we cannot be certain what role these SNPs play in the development of AUD (initiation of drinking, susceptibility to binge drinking, proneness toward harmful alcohol use or maintenance of abuse habits, etc.). Our findings suggest a more robust relationship at later time points, potentially related to the power that increased prevalence of AUD at the older age affords in a statistical analysis. However, it will also be important to investigate whether these genetic markers are linked to maintenance of drinking in adults, relative to early initiation behaviors, using larger longitudinal cohorts, when they become available. Finally, there are limitations with the cohort used for this study. Considering that our cohort is population-based sample of adolescents, the number of problem drinkers is relatively low. Moreover, as the cohorts aged, they reduced in size due to participants leaving the study, diminishing the power of the analyses. Finally, while the results of our replication study were in line with the results of the IMAGEN analysis, our main findings were not significant according to classic standards ($p = 0.05$).

Nevertheless, the present suggests an interaction among various candidate genes relevant to the eCB system in predicting AUD, specifically the *CNR1-MGLL-DAGL* loop and their relationship to 2-AG. Further studies are required to further explore the generalizability of these findings and to understand the psychiatric implications of the results.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data for Imagen dataset and SYS can be made available upon request. Requests to access these datasets should be directed to For Imagen dataset, data can be requested at: https://imagen-europe.com/resources/imagen-project-proposal/. For SYS please address: Dr. Zdenka Pausova [zdenka.pausova@sickkids.ca] and Dr. Tomas Paus [tpaus@research.baycrest.org]. Further details about the protocol can be found at [http://www.saguenay-youth-study.org/].

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Psychiatry, Nursing and Midwifery Research Ethics Subcommittees (PNM RESC), King's College London. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LE conceptualized the analysis, ran the analysis using PLINK1.9, wrote and edited the manuscript. SS helped conceptualize the analysis and ran analyses using the PLINK1.9. DV compiled the SYS cohort data and edited the manuscript. TP helped secure access to the SYS and IMAGEN data, edited the manuscript, and supervised the work. PC and GH supervised the project, helped conceptualize the project, secured access to the data, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.645746/full#supplementary-material

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REVIEW
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Exo- and Endo-cannabinoids in Depressive and Suicidal Behaviors

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Cannabis (marijuana) has been known to humans for thousands of years but its neurophysiological effects were sparsely understood until recently. Preclinical and clinical studies in the past two decades have indisputably supported the clinical proposition that the endocannabinoid system plays an important role in the etiopathogeneses of many neuropsychiatric disorders, including mood and addictive disorders. In this review, we discuss the existing knowledge of exo- and endo-cannabinoids, and role of the endocannabinoid system in depressive and suicidal behavior. A dysfunction in this system, located in brain regions such as prefrontal cortex and limbic structures is implicated in mood regulation, impulsivity and decision-making, may increase the risk of negative mood and cognition as well as suicidality. The literature discussed here also suggests that the endocannabinoid system may be a viable target for treatments of these neuropsychiatric conditions.

Keywords: BDNF, HPA, CB1 receptor, depressive behavior, cannabinoids, suicide

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INTRODUCTION

Humans have been consuming cannabis (marijuana) for more than 5000 years, and different civilizations have utilized it for varied reasons, mostly for hedonic purposes. However, in many cultures, human beings have used it to enhance religious or spiritual experiences, and for its purported medicinal value. In the middle of the 20th century, Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) were isolated. However, the great spur to research on endocannabinoids in the scientific community occurred when the cannabinoid-1 (CB1) receptor was cloned in 1990 (1). The discovery of the endocannabinoid system created considerable curiosity in the psychiatric research community due to its influence on neurobiological processes and neurotransmitter systems in the brain. Due to recruitment of the endocannabinoid system in reward, mood and related motivational processes (2–5), its dysfunction plays an important role in the pathophysiology of mood disorders. This review suggests that the endocannabinoid system is a viable treatment target for depressive and suicidal behavior, and discusses directions for future research.

EXOCANNABINOIDS

Exocannabinoids consist of both natural and synthetic cannabinoids (**Figure 1**). Natural compounds of the cannabis plant are referred to as phytocannabinoids to differentiate them from the synthetic cannabinoids and endocannabinoids. In Atharvaveda, a sacred Hindu religious

scripture (written between 2000 and 1400 BCE), cannabis was referred to as one of the five sacred plants, believed to be a source of happiness, bestower of joy and bringer of freedom (7). Cannabis is a genus of plants in the family "Cannabaceae" that has three common species: Cannabis Sativa, Cannabis Indica and Cannabis Ruderalis (8). Approximately 490 compounds have been identified in the cannabis plant, with more than 70 of them considered "cannabinoids" (9). The best-known and well-studied cannabinoid is Δ^9-THC, which is the primary psychoactive constituent of cannabis. Another major compound, CBD, is a non-psychoactive substance which opposes some of the effects of Δ^9-THC (9). Δ^9-THC is a partial agonist at both CB1 and CB2 receptors, and its psychoactive effects are likely mediated through CB1 receptors by causing an imbalance of GABA/glutamatergic neurotransmission, as well as dopamine release. Its potential immunologic or anti-inflammatory effects are likely mediated *via* CB2 receptors (10, 11). Studies examining the effects and actions of CBD are beginning to emerge. It acts mainly through GPR55 and inhibits some of Δ^9-THC effects *via* antagonistic/negative allosteric modulator activity at the CB1 receptor (7). It also stimulates the vanilloid receptor type 1 (VR1), similar to the effects of capsaicin, and increases arachidonoyl ethanolamide (AEA/anandamide) by inhibiting its uptake and hydrolysis (12).

Cannabis can be smoked, inhaled, mixed with food, made into snacks or drunk as a tea. The bioavailable exocannabinoids and their quantities, therefore, vary widely, depending on form and route of use. Intravenous administration and inhalation have somewhat similar pharmacokinetics and bioavailability. Δ^9-THC and CBD are both highly lipophilic; after inhalation, peak plasma concentration reaches rapidly in few minutes (13). Δ^9-THC has a half-life of about 6 min after the initial use, but long-term use may increase its half-life up to 22 h (14). On the other hand, CBD has a long half-life (16–30 h), and it may increase up to 5 days in daily users (14). Cannabinoids rapidly distribute into well-vascularized organs such as the lung, heart, brain, liver, and later into less vascularized organs. However, with chronic use, cannabinoids will accumulate in adipose tissues (14). Δ^9-THC and CBD are metabolized predominantly in the liver by cytochrome enzymes (15, 16).

ENDOCANNABINOIDS

The endocannabinoid system is a neuromodulator system that consists of two classical G-protein coupled receptors (GPCRs; CB1 and CB2), their endogenous ligands (endocannabinoids) such as AEA and 2-arachidonoyl glycerol (2-AG), and the enzymes and proteins which regulate the levels of endocannabinoids (**Figure 1**). Recent evidence suggests that GPR55 is a purinergic non-CB1/CB2 receptor at which endo and exo-cannabinoids act, and is considered as a putative CB3 receptor (17), although it has very low homology for CB1 (14%) and CB2 (15%) (18). The CB1 receptors are expressed both in peripheral tissues and in the central nervous system. These receptors are considered to be the most abundant GPCR neuromodulatory receptors in the brain, but are also expressed in the spleen, lung, thymus, heart and vascular system (19). The activation of these receptors leads to behavioral and psychoactive effects (1). Whereas CB2 receptors are abundantly expressed in peripheral tissues such as leukocytes, spleen, tonsils, thymus, lungs and testes (20, 21). Studies also reveal the presence of CB2 receptors in the brain (22) especially in microglial cells (23–25). Using reverse transcription polymerase chain reaction, CB2 receptor mRNA expression was found in cerebellum, cortex and brainstem of the rat brain (26). GPR55 is expressed in multiple brain regions, including PFC (prefrontal cortex), amygdala and striatum (27–30), and is shown to dimerize with CB1 receptors in the striatum (31). Recent studies demonstrate that lysophosphotidyl inositol (LPI) and NAGly palmitoylethanolamide (PEA) elicit biological responses through GPR55 (18, 19, 32, 33) suggesting that these two lipids are endogenous ligands for GPR55. Some of the endocannabinoids can also activate GPR55, even though it lacks the classical CB binding pocket, and therefore it is also considered as an atypical CB receptor (34).

Human CB1 and CB2 receptors contain 472 and 360 amino acid residues, respectively. These receptors contain highly glycosylated extracellular amino-terminal and an intracellular carboxyl-terminal connected by seven transmembrane domains (20, 35). The CB1 receptors are coupled to many secondary messenger systems. They are negatively coupled to adenylyl cyclase (AC) and N- and P/Q type Ca^{2+} channels, and positively to A-type and inwardly rectifying K^+ channels and mitogen-activated protein kinases through $G_{i/o}$ proteins (36). The activation of CB1 and CB2 receptors leads to a reduction of cAMP production through the inhibition of AC (37). Conversely, the GPR55 is known to recruit $G\alpha_{12/13}$ for signal transduction to activate phospholipase C (PLC), ERK, mitogen-activated protein kinase and Ca^{2+} release (17, 38, 39). Among endocannabinoids, AEA and 2-AG are extensively studied (**Figure 1**). AEA was the first one to be isolated (40), followed by 2-AG (41). There are several other endocannabinoids, such as LPI, O-arachidonoyl ethanolamine (virodhamine) (42), 2-arachidonoyl glycerol ether (noladin ether) and N-arachidonoyl-amino acids such as N-arachidonoyl dopamine (NADA) (42–46). However, the physiological role of these ligands is yet to be clearly understood. After activation of CB1 receptors on presynaptic membranes, AEA and 2-AG are degraded by enzymatic hydrolysis. The CB1 receptor-mediated retrograde regulation of synaptic strength is required to produce 2-AG whereas AEA is synthesized either during tonic control of synaptic signaling or 2-AG mediated control of synaptic strength (47).

The endocannabinoids produce varied effects due to their different affinities for the CB receptors. 2-AG is a high efficacy agonist for both CB1 and CB2 receptors whereas AEA is a low efficacy agonist at these receptors (48). These ligands are released from postsynaptic neurons in response to increase in intracellular calcium and activation of the Gq/11-linked G-protein-coupled receptors in the postsynaptic region. Upon release, they activate presynaptic CB1 and CB2 receptors, and cause transient, as well as long-lasting reduction in the release of neurotransmitters. The postsynaptic synthesis and release of endocannabinoids, and their activation of presynaptic CB1 receptors, led to the discovery of the retrograde mechanism

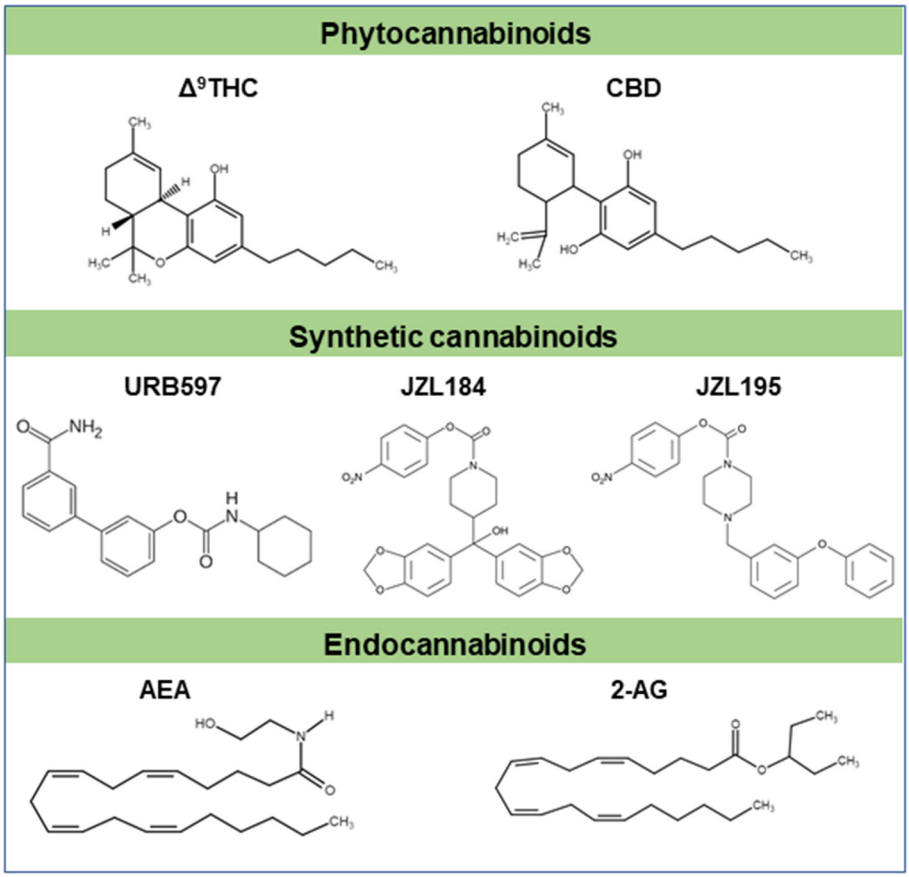

FIGURE 1 | Chemical structures of some cannabinoids. Δ^9-THC and CBD are major plant derived cannabinoids (phytocannabinoids). Synthetic cannabinoids (URB597, JZL184, JZL195 etc) are cannabimimetics. Natural cannabinoids are endogenously produced in humans and other animals are referred to as endocannabinoids (AEA, 2-AG etc). These cannabinoids typically signal through cannabinoid receptors like CB1 and/or CB2 receptors. Part of this figure has been adapted from (6) with permission.

of endocannabinoids in the modulation of neurotransmitter release from presynaptic terminals. Endocannabinoids, unlike classical neurotransmitters, are not stored in the vesicle, but they are synthesized on demand through the hydrolysis of the cell membrane lipid precursors. Several pathways for AEA synthesis have been proposed. However, AEA appears to be mainly produced from N-arachidonoyl phosphatidyl ethanol (NAPE) by the sequential action of N-acyltransferase (NAT) and N-acylphosphatidyl ethanolamine-specific phospholipase D (NAPE-PLD) (49). In immune cells and in the brain, AEA is synthesized via formation of a NAPE phosphodiester bond by a NAPE-selective PLC followed by dephosphorylation of the resulting phospho-AEA to produce AEA (50). In contrast, 2-AG is produced from 2-arachidonoyl-containing phospholipids, primarily arachidonoyl-containing phosphatidyl inositol bis-phosphate. The main biosynthetic pathway involves the hydrolysis of phosphatidylinositol by PLC, producing 1,2-diacylglycerol (DAG), which is then converted into 2-AG by a diacylglycerol lipase α/β (DAGL) (48, 51). AEA is metabolized by hydrolysis of an integral membrane protein fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine. The monoacylglycerol lipase (MAGL) is primarily involved in catabolism of 2-AG by hydrolysis of the ester bond between the arachidonic acid (AA) and glycerol (52, 53).

EXO- AND ENDO-CANNABINOIDS IN DEPRESSIVE BEHAVIOR

Major depressive disorder (MDD) is a debilitating disease that is characterized by depressed mood, diminished interest, impaired cognitive function, and biological symptoms such as disturbed sleep, reduced libido and appetite (54). As the 4th leading cause of disability worldwide, MDD is highly prevalent (55), adversely affects the quality of life, and is significantly associated with mortality. A metanalysis of longitudinal and prospective studies in adolescents reports that use of cannabis increases the risk of developing depression and suicidal behavior in young adulthood (18 to 32 years) (56). Data from Youth Risk Behavior Survey also shows significant increase odds of reporting depression and suicidal thoughts in cannabis users (57). Although robust evidence lacks for increased risk of

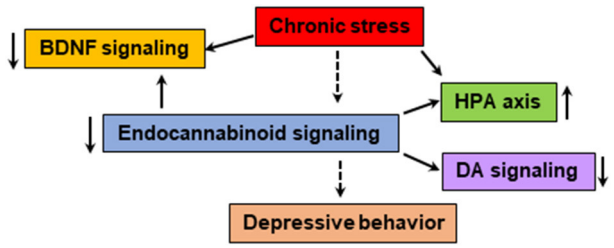

FIGURE 2 | The endocannabinoid system is known to modulate the BDNF, HPA axis and monoamine neurotransmitter systems, which are shown to be impaired in patients with depressive behavior. Chronic stress could dysregulate the function of endocannabinoid-mediated signaling leading to abnormalities in neurogenesis, HPA axis, monoamine systems and immune response. The pharmacological agents that enhance endocannabinoid/CB1-mediated signaling and physical exercise shown to have positive effect on mood.

suicidality in acute cannabis use, chronic cannabis use has been shown to increase the risk of suicidality (58). Its use also markedly increases the risk of depression following a first episode of psychosis (59). Importantly, non-medical use of cannabis in depression was associated with higher suicidal ideation and impeded the improvement of depression (60). However, other studies found no association between depressive behavior or suicidality in cannabis use and the severe course of depressive behavior was attributed to confounding factors (61).

Stress is the most important predisposing factor for depression. Chronic stress exposure in animal models has been shown to downregulate CB1 receptor expression in several brain regions (62, 63). Male rats exposed to chronic unpredictable stress for 21 days resulted in a marked increase in CB1 receptor density as measured using radiolabeled CB1 receptor agonist, CP-55,940 in the PFC and, a reduced density in the hippocampus, hypothalamus and ventral striatum (64). These changes are accompanied with a significant reduction in anandamide levels in almost all the brain regions studied (64) suggesting an overall blunted anandamide-mediated CB1 signaling with specific alteration of the endocannabinoid system in cortical and subcortical brain regions in chronic model of depression behavior. Significant reduction of AEA and 2-AG levels have been reported due to stress but studies also show increase in 2-AG levels (63) suggesting a bidirectional regulation of these two endocannabinoids in certain brain regions. It remains to be examined whether different types and duration of psychological stressors have differential effect on other components of the endocannabinoid system.

The neuroendocrine system plays an important role in the etiology and pathogenesis of mood disorders (65, 66). Chronic stress could reduce endocannabinoid signaling, leading to activation of the hypothalamic-pituitary-adrenal (HPA) axis and suppression of cell proliferation in the hippocampus (**Figure 2**). For instance, stress associated reduction in AEA is facilitated by corticotropin-releasing hormone (CRH) signaling could lead to an increase in AEA hydrolysis by FAAH, especially in the amygdala (11, 63, 67). This dysregulation is thought to be one of the contributing factors for anhedonia (63). Conversely, stress-induced increase in 2-AG, seen primarily in the medial prefrontal cortex (mPFC) and hippocampus, is believed to buffer and reduce the effects of stress on the brain by helping to terminate stress-induced HPA axis activation and promoting habituation to stress (63). Underscoring the role of the PFC, one suggested model entails the effect of steady levels of endocannabinoids fine-tuning GABAergic inhibition through CB1 receptors in the raphe nucleus and the basolateral amygdala. This system may be recruited in times of stress and adversity (63). These studies indicate that the endocannabinoid system plays an important role in terminating the stress response via CB1-mediated suppression of GABA release in the mPFC, likely increasing the activity of the principal neurons of the prelimbic region, which has the effect of suppressing the stress response (68). Such change in ventromedial (vm) PFC is possibly due to stress-induced increase in the levels or activity of FAAH. An increase in FAAH reduces AEA levels and this may cause a compensatory upregulation of CB1 receptor binding sites in the vmPFC in an effort to maximize the diminishing AEA signaling pool to produce mood enhancement (62). The activation of CB1 receptors has also been shown to exert antidepressant-like activity in the rat model using the forced swim test (69). This study found that the administration of AM404 (an endocannabinoid uptake inhibitor), the CB1 receptor agonist HU210 and oleamide exert similar effects as of the antidepressant desipramine. In chronic mild stress paradigm mimicking the physiological response of depression, a selective inhibitor of the FAAH enzyme, URB597 that increases AEA has been shown to exert antidepressant-like effects in Wister rats (70) but such effects were not reported in standard test condition through pharmacological inhibition or genetic deletion of FAAH. However, when methodological changes were made such as using altered ambient light, significant effects on emotional reactivity were observed (71). Taken together enhancement of AEA-mediated signaling appears to reduce depressive behavior (72, 73).

In women with major depression, serum 2-AG levels correlated with the duration of the depressive episode: the longer the duration of the depression, the lower the 2-AG levels. Such changes were not seen in regard to AEA but a strong negative correlation was observed with serum AEA levels and anxiety symptoms in affective disorders (74). A subsequent study found that basal serum concentrations of AEA and 2-AG were significantly reduced in women with major depression (75). Serum 2-AG but not AEA levels were found to increase after the social stress test in the same patients (75). Interestingly, serum levels of other endocannabinoid ligands such as PEA and OEA were also lower in these patients (75). An increase in serum AEA levels with a corresponding reduction in depressive symptoms has also been shown in women with MDD after moderate exercise (76). Though exercise can improve mood through other signaling mechanisms such as serotonin (5-HT) (77), varying levels of aerobic exercise increases AEA and 2-AG (78). Additionally, a 30 min exercise as an adjunctive treatment in substance use disorder elevates AEA level and improves mood (79). Interestingly, exercise increases not only AEA and

2-AG but also lipid messengers OEA, PEA, N-docosahexaenoyl ethanolamine and 2-oleoylglycerol (80) resulting in an acute improvement in pain and mood (81). Moreover, exercise-induced increase in AEA levels elevates BDNF, and influences neuroplasticity and the antidepressant effects (82). Such mood-enhancing properties of endocannabinoids may also play a role in non-suicidal self-injurious behavior, a negative behavior that could reduce the intensity of negative affective states (83). It is important to note that circulating endocannabinoids can be derived from multiple organs, tissues, immune cells etc. (46), and thus the association between endocannabinoid levels in blood and brain is currently not clear. Nevertheless, an intravenous administration of endocannabinoids has been shown to enhance reward-seeking behavior in rats (84, 85) suggesting that circulating endocannabinoids might have central effects to some degree as these lipids can readily cross the blood brain barrier (86) and activate reward and other neuronal processes.

It is well-documented that women are more susceptible to MDD than men (54, 87–89). There have been some attempts to delineate biological causes of this dimorphism, but reasons remain obscure. Studies in animal models have shown that chronic stress could dysregulate the endocannabinoid system differently in males and females. For instance, chronic stress significantly downregulates CB1 receptor levels in the hippocampus of male rats, while it upregulates these receptors in the dorsal hippocampus of female rats (90, 91). Our recent study revealed a decrease in postmortem levels of AEA and 2-AG in the ventral striatum (nucleus accumbens; NAc) of women with MDD as well as in female Wistar Kyoto (WKY) rat, a genetic model of depression (92). This study also found lower levels of BDNF in the ventral striatum of MDD patients. Interestingly, pharmacological inhibition (by use of JZL195) of the two major endocannabinoid degrading enzymes, FAAH and MAGL, elevated both endocannabinoids and BDNF levels in the ventral striatum, and reduced depressive-like behavior in female rats (92). The ventral striatum has been shown to play a central role in reward and motivation related processes that become dysfunctional in mood disorders (93, 94). The deficiency in endocannabinoid-mediated signaling in this brain region may impact reward processing leading to anhedonia, a major symptom of clinical depression. Besides BDNF, dopamine signaling in the ventral striatum plays a critical role in the pathogenesis of MDD (94–96). Both AEA and 2-AG, as well as pharmacological activation of CB1 receptors, elevate dopamine release in the NAc (97, 98) and increase hedonic taste (97). The behavioral effects of JZL195 are most likely associated with endocannabinoid-mediated activation of dopaminergic signaling in the ventral striatum which promotes reward and motivation-related processes. Beside reward deficit, social withdrawal is one of the major symptoms observed in patients with MDD. Recent studies suggest that the endocannabinoid system mediates social behavior in rodent models. Systemic administration of JZL195 has been shown to enhance social interaction in WKY rats (92). The deletion of DAGLα (2-AG synthesizing enzyme) in direct medium spiny neurons of the striatum elicits deficiency in social behavior in mouse (99). Moreover, pharmacological augmentation of 2-AG via administration of JZL184 (MAGL inhibitor), reduces glutamatergic activity at basolateral amygdala-NAc synapse and rescues deficits in social interaction (100). These studies underscore the importance of endocannabinoids in regulating social behavior.

The CB1 agonists and FAAH inhibitors can also enhance central 5-HT and noradrenergic (NE) transmission and promote neurogenesis in the hippocampus (101). Such effects are also observed with most of the currently available antidepressants. In this regard, FAAH inhibitors may have more beneficial effects than CB1 agonists due to the lack of adverse cannabinoid side-effects and a better therapeutic window (101). The antidepressant-like effects of CBD have been shown in genetic animal model of depression (102) suggesting that CBD is a potential for the treatment of clinical depression and anhedonia. Conversely, besides some beneficial effects in certain illnesses like pain and spasticity (103), no strong favorable effects of Δ^9-THC in depressive behavior have been reported. Δ^9-THC can also have short and long-term adverse effects (104, 105). The studies which evaluated the effects of cannabinoids for pain have found no significant difference between cannabinoids such as dronabinol (Δ^9-THC) and nabiximol (Δ^9-THC and CBD) in improving depression compared to placebo when depression was used as an outcome measure (105–107). In fact, nabiximol (Δ^9-THC and CBD) had negative effects on depressive symptoms at higher dose but no difference in improving depression when compared to placebo at lower doses (106). Further detailed studies on use of cannabis products for treating mental illnesses are clearly needed due to lack of well-designed randomized trials and small sample size (108).

THE ENDOCANNABINOID SYSTEM IN SUICIDE AND IMPULSIVITY

Suicide accounts for 1.4% of all deaths worldwide (109). About 30% of individuals with mood disorders die by suicide (110). Up to 87% of suicide victims suffer from major depression, while up to 15% of patients with unipolar depression are most likely to commit suicide (111). When depression is comorbid with alcohol use, the suicide rate increases significantly (112). Impulsive behavior is also an important risk factor for suicide (113). A study conducted in combat veterans with and without a history of suicide attempts observed a higher serum concentration of 2-AG among suicide attempters; in addition, stress-induced cortisol levels positively correlated with 2-AG levels (114). However, AEA levels negatively correlated with suicide ideation scores among attempters (114). Another study that examined victims of the 9/11 World Trade Center disaster found a positive correlation of AEA levels with circulating cortisol (115).

The PFC is necessary for healthy neurocognitive function and the pathogenesis of depression correlates with relative hyperactivity in vmPFC and hypoactivity in the dorsolateral PFC (dlPFC) (116). Another cortical area, the dlPFC plays a primary role in cognitive functions such as working memory,

goal-directed action, abstract reasoning and judgment. These executive functions are significantly affected in depression (117) and suicidality. Increased activity of vmPFC plays an essential role in the generation of negative emotion. Lesions in the vmPFC can cause a loss of self-awareness and insight, with a marked reduction in feelings of shame, guilt, embarrassment, and regret (117). Abnormal activation of the orbitofrontal prefrontal cortex is also shown to increase compulsivity and repetitive behaviors, similar to the behaviors seen in substance use disorders (118). The multiple domains of PFC functional impairment manifest as impaired cognitive control of mood, pessimism, impaired problem solving, over-reactivity to negative social signs, excessive emotional pain, and suicidal ideation (119). These effects cumulatively contribute to an increased risk of suicide. A localized PFC hypofunction in people who have attempted suicide was proportional to the lethality of the suicide attempt (120).

A postmortem study of patients with MDD who committed suicide revealed a higher density of CB1 receptors in the dlPFC (121). Whether or not this elevation in CB1 receptors is due to neuroadaptation to lower levels of endocannabinoid is currently unknown. Both the activity and level of the FAAH enzyme (which degrades AEA) are found to be lower in the ventral striatum of individuals with alcohol use disorder (AUD) when compared to non-psychiatric controls (122). In suicide victims with AUD, the level and activity of FAAH are significantly higher compared to the group with AUD who did not commit suicide (122). These observations suggest that suicide may be associated with upregulation of CB1 receptors in the ventral striatum. While higher levels of endocannabinoids were also found in suicide victims in this study, additional well-characterized postmortem samples are needed to tease out this finding due to potential confounding effect of alcohol use on endocannabinoid system in these subjects. The positive correlation of upregulation of CB1 and FAAH activity indicates that CB1 receptor sensitization in the ventral striatum of suicide victims could be contributed to a decrease in AEA levels. However, an increase in CB1 receptor expression and lower levels of MAGL activity were reported in postmortem PFC of subjects with AUD (123). In addition to the findings related to CB1, density of CB2 receptors and GPR55 gene expression were found to be significantly lower in the dlPFC of suicide victims (124). Nevertheless, higher protein levels of CB2 receptors in both neurons and astrocytes were observed in the dlPFC of suicide victims (124). This suggests that CB2 receptors and GPR55-mediated signaling mechanisms may also play a role in the pathophysiology of suicidal behavior. These findings may have etiologic and therapeutic implications for the treatment of suicidal behavior. In addition to mood disorders, substance use disorders, including alcohol are independently associated with an increase in the risk of suicide (125–127). In this regard, a postmortem study revealed elevated levels of CB1 receptors in the dlPFC of patients with AUD who were suicide victims, compared with patients with AUD who were not victims of suicide (128). This finding that resembles the findings in depressed suicide victims (121), supports the evidence linking sensitization of cortical CB1 receptors to suicide. Higher levels of the endocannabinoids AEA and 2-AG were also observed in the dlPFC of suicide victims who diagnosed with AUD (128) suggesting an endocannabinoid system dysregulation in the PFC of suicide victims.

CONCLUSION AND FUTURE PERSPECTIVES

The endocannabinoid system and its role in psychiatric disorders is a rapidly growing area of research. The evidence discussed in this review supports that the endocannabinoid system is an integral part of the neurobiological processes that regulate reward, stress response and mood. Dysfunction of the endocannabinoid system could contribute to the manifestation of behavioral abnormalities seen in depression and suicidal behavior. It is important to note that depressive disorder comprises a cluster of complex symptoms which has a heterogeneous presentation across patients. Although dysregulation of the 5-HT and NE systems are implicated in the pathophysiology of mood (129), many patients do not adequately respond to existing antidepressants which are targeted to these neurotransmitter systems. While the endocannabinoid system modulates the function of HPA axis, neurotrophic factor (BDNF), and many neurotransmitter systems, including 5-HT, NE and DA, a dysfunction in endocannabinoid system most likely has a greater effect on the functions of these systems and mood related behaviors (**Figure 2**). It is difficult to delineate whether any symptoms specific to dysregulation of the endocannabinoid system or the monoaminergic system predispose to depressive and suicidal behaviors. Thus, mere enhancement or modulation of the endocannabinoid system may not be the best treatment modality. Perhaps therapeutic agents which target one or more of these systems simultaneously might be the goal for future research. There are no conclusive findings until now which clearly demonstrate specific modulatory effects of the endocannabinoid system on suicidal thoughts.

There are inconsistent findings on the role of cannabis in depressive and suicidal behaviors which stem from confounding factors. The reported pharmacologic effects targeting endocannabinoid system also remain somewhat inconsistent, due to lack of selective reagents and use of diverse behavioral paradigms in preclinical studies. Current, advanced neuroscientific techniques can improve understanding of the significance of endocannabinoid signaling, including its effects on regulation of mood and reward processes. New therapeutic agents that act on the endocannabinoid system are most likely to emerge as pharmacologic treatment options for depression and many other disorders. The pharmacologic agents which are available for use in clinical practice need further research into their safety and therapeutic benefits. In addition to the pharmacological strategies, physical exercise is beneficial in elevating the endocannabinoids and mood. Whether circulating endocannabinoids could serve as biomarkers for the diagnosis and prognosis of MDD and other psychiatric disorders need to be determined. It remains to be examined if depressive and suicidal behaviors are

independently related to dysfunction in specific components of the endocannabinoid system. The mechanisms driving the gender and brain region-specific changes seen in these disorders remain unclear, and further studies are warranted in this field.

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Cannabis Affects Cerebellar Volume and Sleep Differently in Men and Women

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Background: There are known sex differences in behavioral and clinical outcomes associated with drugs of abuse, including cannabis. However, little is known about how chronic cannabis use and sex interact to affect brain structure, particularly in regions with high cannabinoid receptor expression, such as the cerebellum, amygdala, and hippocampus. Based on behavioral data suggesting that females may be particularly vulnerable to the effects of chronic cannabis use, we hypothesized lower volumes in these regions in female cannabis users. We also hypothesized poorer sleep quality among female cannabis users, given recent findings highlighting the importance of sleep for many outcomes related to cannabis use disorder.

Methods: Using data from the Human Connectome Project, we examined 170 chronic cannabis users (>100 lifetime uses and/or a lifetime diagnosis of cannabis dependence) and 170 controls that we attempted to match on age, sex, BMI, race, tobacco use, and alcohol use. We performed group-by-sex ANOVAs, testing for an interaction in subcortical volumes, and in self-reported sleep quality (Pittsburgh Sleep Questionnaire Inventory).

Results: After controlling for total intracranial volume and past/current tobacco usage, we found that cannabis users relative to controls had smaller cerebellum volume and poorer sleep quality, and these effects were driven by the female cannabis users (i.e., a group-by-sex interaction). Among cannabis users, there was an age of first use-by-sex interaction in sleep quality, such that females with earlier age of first cannabis use tended to have more self-reported sleep issues, whereas this trend was not present among male cannabis users. The amygdala volume was smaller in cannabis users than in non-users but the group by sex interaction was not significant.

Conclusions: These data corroborate prior findings that females may be more sensitive to the neural and behavioral effects of chronic cannabis use than males. Further work is needed to determine if reduced cerebellar and amygdala volumes contribute to sleep impairments in cannabis users.

Keywords: marijuana, tetrahydrocannabinol, magnetic resonance imaging, sexual dimorphism, subcortical volume

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INTRODUCTION

There are marked sex differences in the acute and long-term effects of drugs of abuse, including subjective effects, neurological impact, and behavioral outcomes. These disparate effects may be due to differences in metabolism, body fat and water distribution, hormones, and sexual dimorphism in brain function. For example, differences in metabolism and bioavailability cause higher blood alcohol levels in females and greater vulnerability to the negative effects of alcohol, compared to males consuming the same amount of alcohol (1, 2). Greater drug effects in females are thought to contribute to "telescoping," the observation that women tend to progress from first use to seeking treatment for cannabis use disorder (CUD) more rapidly than men. This phenomenon has been described across several drugs of abuse, including cannabis use disorders (CUD) (3). Despite this, the prevalence of cannabis use and CUD is higher in males than females (4), which is driven by a greater rate of drug initiation among men than women, though this gap is narrowing (5). Along with this acceleration to CUD, women also experience stronger cannabis withdrawal symptoms than men during periods of abstinence (5), as well as worse outcomes on experimental cannabis therapies such as buspirone (6) and vilazodone (7).

These differences in responses to cannabis are likely related in part to sex differences in the function and structure of subcortical brain regions rich in cannabinoid-type I receptors (CB1-R, the primary receptor target for THC, the main psychoactive component of cannabis), such as the cerebellum, amygdala, and hippocampus (8). For instance, rats repeatedly treated with THC exhibited CB1-R desensitization and downregulation in cerebellum, hippocampus, prefrontal cortex, and striatum, with greater effects in females consistent with the "telescoping" observation (9) and which may be dependent on the estrous cycle (10). Chronic THC treatment also had lasting effects in primates, with THC concentration in the cerebellum approximately double the concentration in blood 24 h after the last dose of THC, indicating that brain regions with high CB1R density can be impacted long after cannabis use (11). Importantly, in individuals with CUD the cerebellum showed significant reductions in brain glucose metabolism during withdrawal whereas its activation during cannabis intoxication was associated with its reinforcing effects (12). Moreover, it has been proposed that the effects of cannabis on the cerebellum are relevant to cannabis addiction (13). As it relates to sex differences brain imaging studies showed that in individuals with CUD, females compared to males showed a blunted metabolic response to a stimulant challenge, which was most prominent in CB1-R-dense regions: cerebellum, hippocampus, and thalamus (14). Sex differences in the brain and behavior of cannabis users may also be critically related to sex differences in sleep quality, which is recognized as a factor impacting long-term outcomes in people with CUD (15). However, very little work has been done to describe the possible neurobiological underpinnings of sex differences in humans with a history of chronic cannabis use.

A broad literature has been devoted to understanding the effects of cannabis use on subcortical brain volumes. Findings have been inconsistent, with some studies finding substantially smaller subcortical volumes in chronic cannabis users compared to controls (16–18), whereas others have reported that after controlling for key confounding variables like tobacco usage, these differences are virtually non-existent (19, 20). We and others have argued that these discrepant findings are due to generally small sample sizes and inadequate matching on control groups (21). Nevertheless, several recent reviews have been devoted to the topic (22–24) and some consensus seems to have emerged that cerebellum, amygdala, and hippocampus volumes appear to be most consistently affected by chronic cannabis use across studies (8). However, whether these differences are moderated by sex, and are associated with behavioral outcomes such as sleep quality remains unknown.

Current findings regarding cannabis use and sleep quality are mixed, particularly when considering sex differences. Previous studies using the Pittsburgh Sleep Quality Index self-report scale (PSQI) among generally healthy adults, reported that women had lower scores on sleep quality (25–28), sleep efficiency (27), and higher sleep disturbances (28) than men, suggesting that sex differences in sleep quality may exist even before taking substance use into account. Chronic cannabis use can further complicate this picture. Acute withdrawal from cannabis can contribute to objective and subjective sleep disturbances, which are more common in chronic users (29, 30). Acutely cannabis can decrease sleep latency, making it easier to fall asleep (31, 32); however, long-term sleep quality is negatively impacted (15). In fact, roughly half of adults with CUD reported that cannabis use had caused them difficulty sleeping in the past 90 days (33). Heavy users also reported a decrease in desirable sleep aftereffects (e.g., restful sleep, duration) over time (34). Females compared to males who had "risky" use of both alcohol and cannabis reported especially poor sleep quality reflected by high PSQI total scores (35), but it was not clear whether alcohol or cannabis use was most associated with this pattern. In sum, while cannabis use and sex can have strong effects on sleep quality, we are not aware of any studies that have investigated the interaction between these two factors. This is particularly relevant given a wide body of work that chronic impaired sleep quality can negatively impact brain structure [e.g., (36)].

Together, converging evidence suggests that there are sex differences in the effects of chronic cannabis use on subcortical brain volumes and sleep. However, the interaction of sex on cannabis effects on subcortical brain volumes and sleep quality has not been investigated. To address this neglect, we took advantage of Human Connectome Project data (37) to examine brain structure and sleep quality in a relatively large number of participants with a history of chronic cannabis use and well-matched controls. We hypothesized that female cannabis users would have smaller volumes in amygdala, hippocampus, and cerebellum, which are subcortical regions dense with CB1-Rs (38), and poorer sleep quality than male cannabis users.

MATERIALS AND METHODS

Participants

Participants included in this study provided written informed consent at Washington University in St. Louis (39). Out of 1,005

TABLE 1 | Demographics and clinical characteristics for chronic cannabis users (CAN) and controls (CTL).

	Mean (SD)	Mean (SD)	M vs. F: *T-stat, p*	CAN vs. CTL: *T-stat, p*
Cannabis (CAN)	**Males ($n = 114$)**	**Females ($n = 56$)**		
Age	27.614 (3.635)	28.714 (3.944)	−1.754, 0.082	−0.247, 0.805
BMI	26.033 (4.110)	27.477 (6.320)	−1.556, 0.124	−0.284, 0.777
Edu	14.465 (1.825)	14.018 (1.995)	1.411, 0.161	−2.039, 0.042
Tobacco use (Composite-Z)	0.647 (1.082)	0.366 (1.096)	1.578, 0.118	3.246, 0.001
Alcohol use (Composite-Z)	0.218 (0.424)	0.061 (0.341)	2.600, 0.010	0.901, 0.368
% Caucasian	72.81	57.14	$\chi^2 = 4.210$, $p = 0.040$	
% Black/African American	15.79	30.36	$\chi^2 = 4.874$, $p = 0.027$	
Controls (CTL)	**Males ($n = 114$)**	**Females ($n = 56$)**		
Age	27.658 (3.604)	28.929 (3.756)	−2.101, 0.038	
BMI	26.406 (3.953)	27.163 (5.048)	−0.976, 0.332	
Edu	14.702 (1.755)	14.768 (1.849)	−0.223, 0.824	
Tobacco use (Composite-Z)	0.189 (0.987)	0.191 (0.976)	−0.015, 0.988	
Alcohol use (Composite-Z)	0.184 (0.376)	0.018 (0.249)	3.431, 0.001	
% Caucasian	74.56	58.93	$\chi^2 = 4.323$, $p = 0.038$	
% Black/African American	15.79	30.36	$\chi^2 = 4.874$, $p = 0.027$	

individuals with structural MRI data in the Human Connectome Project, we identified 170 individuals meeting DSM-IV criteria for lifetime (current or prior) CUD and/or >100 lifetime cannabis uses, and without comorbid current or prior alcohol dependence, as in our previous work (21, 40), which became the cannabis group (CAN). We also selected a control group (CTL; $n = 170$) with <10 lifetime cannabis uses, and used the matchControls package in R to try and match controls with the CAN group on: age, sex, education, BMI, race, and a composite measure reflecting past/current alcohol usage (41, 42). Of note, we could not match on tobacco usage, which was higher in the CAN group ($p < 0.001$), and subsequent analyses were performed to ensure results were not driven by past/current tobacco usage. For more details on participant demographics see **Table 1**.

MRI Image Acquisition and Preprocessing

Scans were collected using a custom-made Siemens Connectom Skyra scanner with a 32-channel head coil. T1- and T2-weighted anatomical scans were acquired at 0.7 mm isotropic resolution (37). Structural images were "minimally preprocessed" by HCP investigators through standardized pipelines (43). Images were corrected for gradient non-linearity-induced distortions, readout distortions, and intensity inhomogeneities, and then aligned to the MNI atlas. Then, images were processed through a customized version of Freesurfer. We used the volume values for all subcortical regions (averaged across the left and right regions, where possible) in the Desikan-Killany parcellation (44), which resulted in the analysis of 10 regions: Amygdala, Hippocampus, Putamen, Caudate, Nucleus Accumbens, Thalamus, Pallidum, Brainstem, Cerebellar Cortex, and Cerebellar White Matter.

Self-Reported Sleep Quality

The Pittsburgh Sleep Quality Index (PSQI) was developed in 1988 to assess sleep via 19 questions; it produces a validated global score based on seven sub-scores such as efficiency, quality, and disturbances (45).

Statistical Analyses

Analyses were performed in R version 3.6.2 and in GraphPad Prism version 8.0.1. To test for sex differences in subcortical regional volumes, we constructed linear regression models using the lm Function in R (equivalent to an analysis of variance), where the main effects of sex and cannabis group membership (and their interaction) were the predictor variables, tobacco usage and total intracranial volume were covariates, and each region's subcortical volume was the outcome variable. To correct for multiple comparisons across all 10 regions of interest, we used false discovery rate (Benjamini-Hochberg) correction. We also tested for differences in self-reported sleep quality using the same analytical approach, except that total PSQI score was the outcome variable.

To attempt to link any of the above findings that showed significant cannabis group-by-sex interactions, we performed mediation analysis. We tested whether sleep scores mediated the association between sex and subcortical volumes, using the causal mediation analysis toolbox in R (46) with 1,000 permutations. We also tested the reverse mediation analysis: that subcortical volumes mediated the association between sex and self-reported sleep quality. In these analyses we used only the data from participants in the CAN group ($n = 170$), and we controlled for tobacco usage and total intracranial volume.

Finally, we tested if any of the subcortical volumes or self-reported sleep quality with significant cannabis group-by-sex interactions were driven by participants who had an earlier age of cannabis use onset, since this has been associated with poorer outcomes in cannabis users generally, and in our prior study with differences in subcortical function (42). The HCP recorded age

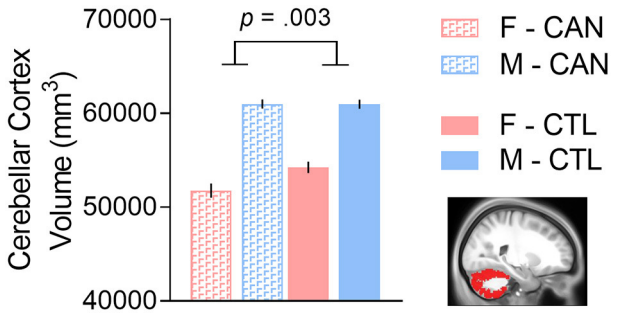

FIGURE 1 | There was a cannabis group-by-sex interaction in cerebellar cortex volume, such that females with a history of chronic cannabis use had smaller volumes than the other groups. We controlled for tobacco usage and total intracranial volume in the analysis. F, Female; M, Male; CAN, Chronic cannabis use group; CTL, control group.

TABLE 2 | Summary statistics for analysis of subcortical regional volumes, showing: (1) the main effects of group, i.e., the chronic cannabis use group (CAN) vs. controls (CTL); (2) the main effect of sex, i.e., Males (M) vs. Females (F); and (3) their interaction.

Region	Group: CAN > CTL t (p_{adj})	Sex: M > F t (p_{adj})	Interaction: t (p_{adj})
Cerebellar cortex	**−3.353 (0.009)**	1.82 (0.232)	**3.699 (0.003)**
Cerebellar WM	−0.835 (0.763)	1.126 (0.473)	1.323 (0.374)
Amygdala	**−2.611 (0.047)**	1.884 (0.232)	1.78 (0.374)
Hippocampus	−1.341 (0.603)	1.228 (0.473)	1.434 (0.374)
Putamen	0.623 (0.763)	1.85 (0.232)	−0.1 (0.921)
Caudate	−0.422 (0.842)	−0.974 (0.473)	0.413 (0.756)
Accumbens	−1.101 (0.679)	1.059 (0.473)	1.446 (0.374)
Thalamus	−0.266 (0.871)	0.379 (0.881)	0.681 (0.633)
Pallidum	0.731 (0.763)	−0.07 (0.998)	0.665 (0.633)
Brainstem	0.162 (0.871)	−0.003 (0.998)	0.911 (0.605)

All p-values are adjusted using False Discovery Rate Benjamini-Hochberg Correction. WM, White Matter. Bold values denote statistical significance ($p < 0.05$).

of first cannabis use on an ordinal scale (1: <14 years old, 2: 15–17 years old, 3: 18–20 years old, 4: 21+ years old). We therefore tested for interaction effects by performing sex-by-age of first use ANOVAs, using only the data from participants in the cannabis use group ($n = 170$), again controlling for tobacco usage and total intracranial volume.

RESULTS

We constructed linear models to determine if the interaction of sex and chronic cannabis usage was significantly associated with subcortical volumes, controlling for tobacco usage and total intracranial volume. We first noted that there were no significant main effects of sex in any of the 10 regions tested after FDR correction (all p's > 0.20). There was a main effect of group in the cerebellar cortex [$t_{(1,334)} = -3.353$, FDR-corrected $p = 0.008$], which was driven by the female cannabis users having lower cerebellar volumes than the other participants [interaction effect: $t_{(1,334)} = -3.699$, FDR-corrected $p = 0.002$; **Figure 1**]. There was also a trend for a main effect of group in the amygdala [$t_{(1,334)} = -2.611$, FDR-corrected $p = 0.047$], with CAN having lower amygdala volumes than controls, but the sex interaction effect was not significant. No other region (including amygdala) showed a significant group or interaction effect (all p's > 0.35; for full results, see **Table 2**).

We further tested whether the interaction of sex and chronic cannabis usage was associated with self-reported sleep quality, again controlling for tobacco usage and total intracranial volume. There was no significant main effect of sex [$t_{(1,334)} = 1.323$, $p = 0.187$], however there was a main group effect [$t_{(1,334)} = 3.233$, $p = 0.001$], which was also driven by the female cannabis users having poorer sleep quality than the other participants [interaction effect: $t_{(1,334)} = -2.208$, $p = 0.028$; **Figure 2**]. In exploratory analysis, we tested whether cerebellum volume was correlated with self-reported sleep quality among the female cannabis users only, but did not observe a significant effect: $t_{1,52} = 0.418$, $p = 0.677$.

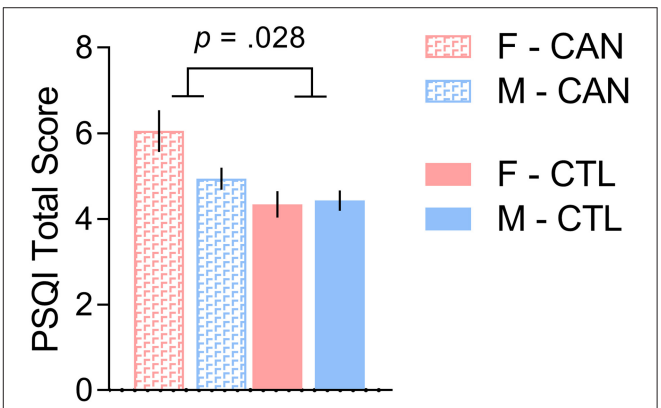

FIGURE 2 | There was a cannabis group-by-sex interaction in self-reported sleep quality, such that females with a history of chronic cannabis use reported more sleep problems than the other groups. We controlled for tobacco usage and total intracranial volume in the analysis. F, Female; M, Male; CAN, Chronic cannabis use group; CTL, control group; PSQI, Pittsburgh Sleep Quality Index.

However, there were no significant mediation effects in the models we tested. Among the cannabis group only ($n = 170$), sleep scores (total PSQI score) did not significantly mediate the sex differences in cerebellar volume: [mediation effect estimate = 15.20, 95% CI = (−208.0, 202.6), $p = 0.93$; direct effect estimate = 4,950, 95% CI = (3,030, 6,890), $p < 1 \times 10^{-16}$]. Likewise, in the reverse model, cerebellar volumes did not significantly mediate the sex differences in sleep scores: [mediation effect estimate = −0.064, 95% CI = (−0.607, 0.470), $p = 0.82$; direct effect estimate = −0.510, 95% CI = (−2.045, 0.980), $p = 0.58$].

Finally, we tested if the significant interaction results in cerebellum volume and sleep were driven by female participants who had an earlier age of cannabis use onset in the CAN group ($n = 170$). The cerebellar volumes showed significant main effects of sex [$F_{(1,160)} = 168.764$, $p < 1 \times 10^{-16}$] and age of first cannabis

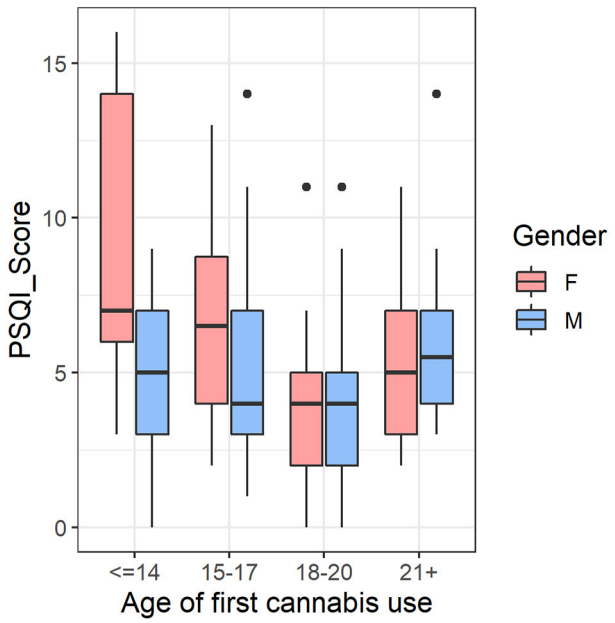

FIGURE 3 | In the cannabis group (CAN) ($n = 170$), there was an age of first use-by-sex interaction in self-reported sleep quality, such that females with earlier age of first cannabis use tended to have more self-reported sleep issues, whereas this trend was not present in male cannabis users. We controlled for tobacco usage and total intracranial volume in the analysis. F, Female; M, Male; PSQI, Pittsburgh Sleep Quality Index.

use [$F_{(3,160)} = 3.812$, $p = 0.011$] but their interaction was not significant [$F_{(3,160)} = 1.583$, $p = 0.196$]. However, for total PSQI score, we observed significant main effects of sex [$F_{(1,160)} = 5.179$, $p = 0.024$], age of first use [$F_{(3,160)} = 4.077$, $p = 0.008$], and their interaction, [$F_{(3,160)} = 3.587$, $p = 0.015$], such that females with earlier age of first cannabis use tended to have more self-reported sleep issues, whereas this trend was not present in male cannabis users (**Figure 3**).

DISCUSSION

Our investigation of the impact of cannabis abuse on various subcortical regions yielded results that add to a body of recent work using Human Connectome Project (HCP) data. For instance, recent cannabis use in this sample was negatively associated with hippocampal volume (47) and smaller left hippocampal volume mediated the association between frequency of cannabis use and working memory deficits in cannabis users (48). Additionally, HCP data has revealed an effect of THC exposure on amygdala microstructure organization (49). In line with these studies, we found that cannabis users had marginally smaller amygdala volumes than non-users. However, we only found a cannabis use-by-sex interaction in cerebellar cortex volumes, suggesting that females may be particularly susceptible to the effects of chronic cannabis use in this region. Finally, we observed that female cannabis users had poorer self-reported sleep quality than the other groups, which was particularly pronounced among females who began using cannabis in early adolescence. We discuss these findings in more detail below.

The cerebellum has traditionally been studied for its role in balance and motor coordination (50), nociception (51), and motor cognition (52, 53). Brain imaging studies in humans have shown that the cerebellum is sensitive to the acute and chronic effects of cannabis (8), including glucose metabolic activity (12, 54), volume, and resting-state activity (13, 55–57). Postmortem studies have found striking differences in the cerebellar structure of drug abusers relative to controls; one group showed increased autophagy biomarkers in the cerebellum of multi-substance drug abusers (58), while another found signs of neurodegeneration in the cerebellar cortex of people who were dependent on opioids, suggesting that drug addiction can negatively impact cerebellar structure (59). Recently, Gil-Miravet et al. found that the cerebellum modulates drug-cue associative memory in cocaine users (60), while Hung et al. showed increased functional connectivity between the pallidum and cerebellum of ketamine users, suggesting that the cerebellum has a fundamental role in the pathophysiology of addiction (61). The cerebellum is clearly affected by cannabis use as well; chronic cannabis users can experience cerebellar-dependent motor adaptation impairment (62), while synthetic cannabinoid users show reduced gray matter volume in the left cerebellum (63). These studies are consistent with several recent reviews published on the topic which note the cerebellum's role as a nexus between motor, reward, and cognitive processes crucial to drug seeking behavior (64–66). Compared to other brain regions, there is a relatively high concentration of CB1-Rs in the cerebellum (38, 67, 68). PET studies have shown that CB-1Rs are reversibly downregulated in people with a history of chronic cannabis consumption, which is likely to contribute to tolerance and dependence with repeated use (69). Previous studies have had mixed findings on the relationship between chronic cannabis use and cerebellar volumes with some studies suggesting that cannabis actually increases gray matter volumes (57, 70–73). Here we found that the smaller cerebellar cortical volumes in cannabis users relative to the controls were driven by the female cannabis users. This could explain the discrepancies in the literature since sex was not accounted for in prior investigations. Indeed, studies finding larger cerebellar volumes in cannabis users had very few or no female participants in the cannabis group: Wang et al.: 25% female (5F/15M); Cousijn et al.: 36% Female (12F/21M); Battistella et al.: 0% Female (0F/31M); Wu and Yang: 25% female (5F/15M); Koenders et al.: 25% female (5F/15M). Those findings contrast with the results in our 33% Female sample (56F/114M) and those of another related study (50% Female; 13F/13M) that found lower cerebellar microstructural integrity in adults at risk for CUD relative to controls (56). These findings underscore the importance of including an adequate number of female participants and of investigating sex differences in brain and behavioral outcomes for people with chronic substance use, for such differences appear to be prevalent throughout the addiction endophenotype (8, 74, 75).

We also observed a trend for a group effect on amygdala volume, with lower volumes in cannabis users compared to healthy controls, in agreement with prior studies (73, 76), and

which correlated with amount of cannabis used and dependence severity (70, 73). In terms of sex differences, one study found that while adolescent female cannabis users had larger right amygdalar volumes than healthy controls, there was no such difference in males (77). However, our finding of lower amygdalar volume in cannabis users was not sex-dependent and likely would not contribute to the sex-dependent impairment in sleep quality that we observed. Nonetheless, it is possible that a deficit in amygdala volume could contribute to the overall poorer sleep quality observed in CUD compared to controls. The amygdala has been previously implicated in poor sleep quality; while functional connectivity between the amygdala and premotor cortex is negatively associated with sleep quality (78), it appears that sleep quality might modulate amygdalar functional connectivity and not vice versa (79). Additionally, patients with narcolepsy have lower GM volume in the amygdala relative to controls, suggesting a possible unidirectional relationship between sleep quality and amygdala volume (80).

Our finding of sex-specific differences in cerebellar volume among cannabis users was not present in other regions with high CB1-R density, such as the amygdala and the hippocampus. Preclinical studies in rats have shown that chronic THC caused downregulation and desensitization of CB1-R in cerebellum, and these decreases were especially large in females (9). Other studies have reported higher baseline CB1-R density in female compared to male rats, although the cerebellum was not examined (10). Human PET studies have similarly found that females have higher baseline CB1-R availability than males in many brain regions (81), including cerebellar cortex (82). Given that CB1-R density was influenced by the estrous cycle in preclinical studies, it is possible that female sex hormones play a role in sex differences in CB1-R availability as well as sex differences on cannabis effects in brain and behavior (10). Animal models could be used to test if sex differences in CB1-R density prior to and after chronic THC exposures may confer female vulnerability to potential neurotoxic effects of cannabis on cerebellar structure and function. Additionally, while initial human PET studies found that CB1-Rs in both sexes were downregulated in response to chronic cannabis use (69), future longitudinal studies should examine whether there are sex differences on the association of CB1-R downregulation with the severity of CUD. This is especially important given that there are currently no FDA-approved pharmacological treatments for CUD, and that one promising candidate, the fatty-acid amide hydrolase (FAAH) inhibitor PF-04457845, was recently shown to reduce cannabis withdrawal severity and promote abstinence, but only men with CUD were included in the trial (83). Thus, much work remains to be done to see if treatments show similar improvements in females and if they do so in part via cerebellar mechanisms. Our group has previously proposed that downregulation of CB1R in subjects with cannabis dependence might increase vulnerability to cortical thinning, suggesting that CB1R availability can lead to structural changes in the brain (21). Another study found that some heavy cannabis users have a genetic predisposition toward cannabis dependence due to a functional single nucleotide polymorphism affecting cannabis receptor-1 gene expression; among cannabis users, minor relative to major allele carriers had lower volume in the nearby hippocampus, but not the amygdala (84). However, cerebellum volume was not examined in this study, and it is possible that there is a similar connection between CB1R and cerebellar volume. Future studies should examine this possibility to uncover the link between CB1R activation/availability and amygdalar/cerebellar volume.

We also observed that females' self-reported sleep quality (as indexed by the global PSQI score) was similarly more vulnerable to the negative effects of cannabis use than for males. Given that patients with degenerative diseases of the cerebellum such as cerebellar ataxia commonly report sleep disturbances, poor subjective sleep quality, restless leg syndrome, and REM behavior disorder, it is plausible that the cerebellar volume loss in female cannabis users contributed to their poor sleep quality (85, 86). However, in our study the effects of cannabis on cerebellar volumes did not mediate the effects of cannabis on sleep quality, which is likely to reflect a more complex association between cannabis effects in brain structure and function. Similarly, the effects of cannabis on sleep quality did not mediate its effects on brain volume, which might also indicate distinct neurobiological processes underlying these two effects. Given the observational nature of this study, we are unable to rule out the possibility that women to start with had lower sleep quality than men as has been reported by other studies (25–28), though in our current study sleep scores in control males did not significantly differ from those in control females. It is also possible that a mismatch between expectation and reality in how cannabis helps with sleep may play a role in self reports; in a majority female (67%) sample of cannabis users, while both frequency and presence of cannabis use were associated with the expectation of improved sleep, cannabis use was actually associated with poorer subjective sleep quality (87). Finally, we observed that females who reported first using cannabis in early adolescence tended to report the worst sleep quality, which aligns with a recent large-scale twin study ($n = 1{,}656$) that reported that regular cannabis use at a young age correlated with shorter sleep duration in adulthood (88). Given the differences in socialization, development, and expectations associated with cannabis use, women may be more vulnerable to the negative sleep effects of cannabis abuse at younger ages than men. These data complement a large body of literature suggesting that early-onset cannabis use is strongly associated with poor neuropsychiatric outcomes (89), and again highlight sex differences as an important future avenue of investigation.

Limitations

The HCP provides a large, high-quality dataset of MRI-based and behavioral data (90). Nonetheless, given that scans were completed between 2012 and 2015 by the WU-Minn Consortium, in Missouri and Minnesota, where medical marijuana was not legalized until 2014 (albeit restrictively and only for certain chronic conditions) it is likely that most participants used cannabis recreationally, not as prescribed by a doctor. While this allows us to compare a uniform population of chronic recreational users to non-users, we were unable to investigate any effects of medical cannabis use on sleep quality or cerebellar volumes. We also do not have any information on whether participants were using cannabis to self-treat sleep

issues. It is possible that when used for medicinal purposes and with low-THC strains that are less likely to lead to CUD (91), cannabis may not have a negative impact on sleep (15). Additionally, while we matched the chronic cannabis use group with controls on several important demographic variables including a composite score reflecting current and past alcohol consumption, cannabis users did not match controls on measures of tobacco usage. Considering that females had lower nicotine use than males and yet they showed greater effects than males, and that we covaried for tobacco use, it is likely that the effects on sleep and cerebellar volumes reflect cannabis and not nicotine effects. Nonetheless we cannot completely rule out that the interaction between cannabis and tobacco use contributed to the effects in brain and sleep quality. Finally, this analysis is limited by the imbalance in the number of male and female subjects in our sample: each one of the groups had twice as many males as females. This sex imbalance is representative of the U.S. population at large, since the majority of people who use cannabis are male (92–98), though this imbalance may affect our results on group differences between cannabis users and controls (as noted in the discussion) and limit our statistical power. This limitation emphasizes the need to include equal numbers of men and women in clinical studies, so that sex differences can be rigorously examined.

Future Directions

Future studies should include polysomnography measurements or other objective measures of sleep architecture and duration in addition to self-reported sleep data. The impact of cannabis use on sleep requires further exploration, for a recent meta-analysis reported that most of the prior studies reported sleep as a secondary outcome and were done on small sample sizes using unvalidated measures (99). Further, studies on the effects of cannabis on sleep architecture and its response to treatment are sorely needed. Finally, future studies should attempt to account for THC potency and a richer quantification of doses and frequency of cannabis use (100), to discern the effects of light vs. heavy cannabis use in general and in the context of these sex-dependent effects on sleep and cerebellar volume.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Washington University in St Louis IRB. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KM, PM, G-JW, and NV: study conception. KM, PM, and DT: data analysis. KM: first draft of manuscript. All authors: editing.

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OPINION
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Endocannabinoids and Precision Medicine for Mood Disorders and Suicide

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INTRODUCTION

Diagnostic precision, prediction and prevention of psychiatric disorders, including major unipolar depression, post-traumatic stress disorder (PTSD) and suicide ideation and attempts, remain underdeveloped areas in psychiatry given a general lack of biomarker assessment in the field. This makes the unmet goal of developing a precision medicine for these debilitating conditions an urgent necessity of neuropsychopharmacology research. Precision medicine, defined as "an emerging approach for treatment and prevention that takes each person's variability in genes, environment, and lifestyle" into account (1), will permit choosing the right treatment for the right person at the right time based on a unique individual neurobiologic biosignature. Indeed, it is anticipated that biomarker discovery will tremendously enhance refinement of individualized medicine that currently rely on subjective symptom assessment based on the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V). Both depression and PTSD are highly prevalent conditions affecting 3.4–12% of the general population and one of the main causes of disability. In the United States, pre-Covid suicide rates have increased by 25–30%, (from 10.5 to 13 per 100,000). These disorders share a number of symptoms and are highly comorbid. MDD is characterized by sadness, anhedonia, disturbed concentration, while PTSD symptoms include avoidance of traumatic memories, hyperarousal, hyperreactivity, flashbacks and nightmares. Antidepressant treatment with SSRIs (the gold standard for PTSD and depression) improves symptoms to about half of patients (2). Developing reliable biomarkers entails the promise of predicting the best treatments for subjects that are more likely to respond to an individually-designed rather than to a "one-fit-all" treatment. Biomarker discovery will also enhance diagnostic evaluation of patients who suffer from psychiatric disorders that share a large symptom overlap and prevalent disorder comorbidity. In recent years, several novel biomarker candidates for mood disorders have been suggested [reviewed in (3)]. The endocannabinoid system has received much interest owing its role in several physiological and pathophysiological functions, including regulation of emotional behavior, cognitive processes, inflammation, chronic pain, epilepsy, and in general, its role underlying neuropsychiatric disorders (4, 5).

This opinion article will focus on the intriguing role of the endocannabinoid system in the regulation of affective disorders, specifically on major depressive disorders, PTSD and suicide behaviors. Furthermore, it will analyze whether data gathered in this exciting area of psychiatric research entails new leads in establishing novel biomarkers for these debilitating and prevalent psychiatric conditions that affect millions worldwide.

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THE ENDOCANNABINOID AND ENDOCANNABINOID-LIKE SYSTEMS AND STRESS RESPONSE

The endogenous cannabinoid system includes the widely investigated anandamide (AEA), that acts as a partial agonist for the cannabinoid receptor type 1 (CB1) and type 2 (CB2) (6), and 2-arachidonoyl-glycerol (2-AG), which acts as a full agonist for both these receptors (7). Both endocannabinoids are synthesized and released from post-synaptic terminals and traffic retrogradely to act at presynaptic CB1/CB2 receptors (8). The biosynthetic enzymes involved in their production and metabolism are the fatty acid amide hydrolase (FAAH) for AEA (9) and monoacylglycerol lipase (MAGL) for 2-AG (10) (**Figure 1**). CB1 is heavily expressed in brain areas devoted in the regulation of stress responses and emotions, which include the prefrontal cortex, ventral hippocampal regions and the basolateral amygdala (16). Mechanistically, CB1 and CB2 receptors inhibit the presynaptic release of neurotransmitters, including GABA and glutamate (17, 18). This action has notoriously been associated with the regulation of anxiety exerted by endogenous and synthetic cannabinoids. In preclinical studies, several CB1 agonists show anxiolytic effects (19), however, this anxiety-like pharmacological effect show a bimodal action, becoming anxiogenic at higher doses (20). Intriguingly, increasing the levels of AEA by genetic deletion of FAAH or using pharmacological FAAH inhibitors (URB597) ameliorates anxiety-like behavior (21). This finding is supported by data showing treatment with rimonabant (SR141716), a selective CB1 inhibitor, increases anxiety and depression (22).

In addition to AEA and 2-AG, endocannabinoid-like modulators include the ethanolamine-derivative N-palmitoylethanolamine (PEA) and its congener, oleoylethanolamide (OEA) (23). PEA is produced by the biosynthetic action of the enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), and, like AEA, is metabolized by FAAH and, more specifically, by N-acylethanolamine acid amide hydrolase (NAAH) (23). PEA is the endogenous modulator of the transcription factor/nuclear receptor, peroxisome proliferator-activated receptor (PPAR)-α, that after heterodimerizing with retinoid X receptor-α, modulates the expression of target genes (24). Like CB1, PPAR-α is expressed throughout the brain, including hippocampus, amygdala and prefrontal cortex (25), and implicated in a host of physiological and pathological processes, including, neuronal differentiation, inflammation, mitochondrial and proteasomal dysfunction, oxidative stress, and neurodegeneration (26).

Stress affects the endocannabinoid system and metabolite levels in opposite directions. While acute stress increases 2-AG, it reduces AEA by enhancing FAAH activity (27–29). Accordingly, preclinical studies show that chronic stress reduces the concentrations of AEA in the amygdala-hippocampal-cortico-striatal circuit (30). These findings support the notion that these endocannabinoids are implicated in distinct neurobiological processes. The role of PEA and PPAR-α on stress response is less studied and understood. However, evidence shows that stress induces a fast FAAH activation resulting in AEA and PEA level reductions (27, 29). PEA levels also decrease when rodents are exposed to predator stress –a model of PTSD (31), and increase after short-term stress in humans (32). Similarly to fluoxetine, administration with PEA induces antidepressant pharmacological effects (11, 33) and pharmacological inhibition of PEA degradation or its biosynthesis upregulation also yields improvement of depressive-like behavior (34–36).

ROLE OF ENDOCANNABINOIDS IN MOOD DISORDERS AND SUICIDE

The endocannabinoid system has been implicated in the neuropathophysiology of stress-related neuropsychiatric disorders (37), however, the role of endocannabinoids in mood disorders is sparse and limited. Among individuals with PTSD, evidence shows a dysregulation in the endocannabinoid signaling. For example, reduced levels of AEA are linked with depression and PTSD (20, 38) and a down-regulation of peripheral AEA levels is associated with an up-regulation of CB1 in brain (39). A genetic polymorphism in the human gene encoding FAAH is implicated in the dysregulation of FAAH-mediated AEA hydrolysis. This drives to a peculiar endophenotype that is associated with reduced index of trait anxiety and enhanced cortico-amygdala connectivity (40, 41). Clinical studies also show the involvement of an abnormal function of the endocannabinoid system in suicide subjects. For instance, evidence shows higher CB1 and CB1-mediated G-protein activation in depressed suicide dorsolateral prefrontal cortex (DLPFC) (13). These findings were also mirrored by studies of alcoholic suicide victims that have evidenced elevated CB1 activation and increased AEA and 2-AG levels in the DLPFC (14). Hence, these similarities between depressed suicide and alcoholic suicide victims point to a role for the endocannabinoid system in suicide in alcoholism and depression. Other studies have showed that CB1 expression is elevated in the ventral striatum of alcohol-dependent suicide subjects (15). Both FAAH expression and activity increased in suicide post-mortem brain (15), which underlay profound abnormalities of the endocannabinoid system. The observation that elevated CB1–mediated signaling in DLPFC of depressed subjects who died by suicide together with the elevated levels of endocannabinoids and CB_1 receptor function strongly supports a hyperactive endocannabinoid system. Whether these are adaptation mechanism remains to be further clarified. However, in some post-mortem studies that include comorbidity with suicide, it is challenging to prove a given neurobiological parameter is linked to the pathophysiology of suicide alone.

In a cross-sectional study comparing morning serum concentrations of AEA, 2-AG but also that of the endocannabinoid-like congeners, PEA and OEA in 30 suicide attempters and 12 psychiatric controls found that, in the morning, AEA and PEA serum levels were increased in suicide attempters compared to controls, unrelated of cannabis use. When cannabis use was controlled in the urine and accounted in

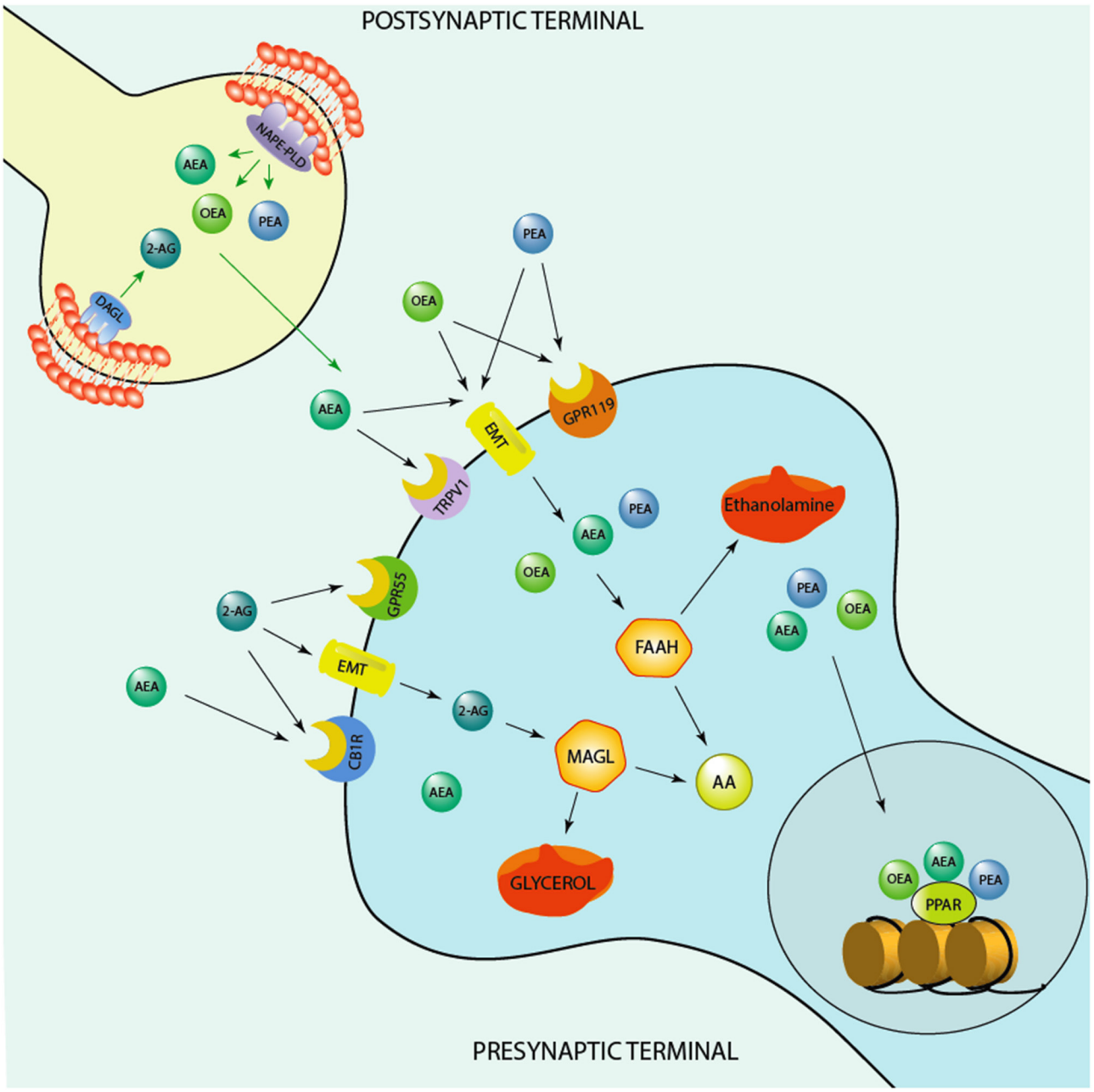

FIGURE 1 | Schematic representation of the endocannabinoid system. Depicted are several biosynthetic and degradation pathways as well as endocannabinoid receptors that are involved in the action of the endocannabinoids, anandamide (AEA), 2-Arachidonoylglycerol (2-AG), and of the endocannabinoid-like ethanolamines, oleoylethanolamide (OEA) and N-palmitoylethanolamine (PEA). AEA, PEA, and OEA share the similar biosynthetic pathway after originating from membrane's phospholipids are synthesized post-synaptically by the action of the enzyme, N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD). 2-AG is instead produced by the action of the enzyme, diacylglycerol lipase (DAGL), prior to be secreted by post-synaptic terminals and act at pre-synaptic cannabinoid receptor type 1 (CB1) and G protein-coupled receptor 55 (GPR55). AEA, PEA, and OEA can act at membrane receptors or be taken up pre-synaptically through endocannabinoid membrane transporters (EMT). They can be degraded by the action of the enzyme, fatty acid amide hydrolase (FAAH) into ethanolamine and arachidonic acid (AA) pre-synaptically. These endocannabinoids influence each concentration by competing for the catalytic action of FAAH. For instance, increased levels of AEA can compete for the catalytic action of FAAH and thereby result in an increase of PEA and OEA levels or vice versa, PEA and, mostly, OEA by competing for FAAH catalytic action may increase AEA levels. PEA may also decrease FAAH expression and thereby elevate its own and the levels of OEA and AEA. 2-AG is instead degraded by monoacylglycerol lipase (MAGL) to glycerol and AA. While OEA and PEA fail to bind to the classic CB1 and CB2, they can influence the action of AEA at transient receptor potential channels of vanilloid type-1 (TRPV1). PEA may activate peroxisome proliferator-activated receptor-alpha (PPAR-α) as well as TPRV1. What makes the endocannabinoid system attractive for developing novel biomarkers concerns the fact that it is constituted by several components, including synthesizing and degrading enzymes to receptors and endogenous modulators and it is widely distributed in the brain. These neuromodulators are implicated in several mechanisms that regulate neuronal functions, including cognition and emotional behavioral regulation. Likewise, synthetic agents that stimulate endocannabinoid receptors or

(Continued)

FIGURE 1 | act on the degrading/biosynthetic enzyme constitute a valid pharmacological approach for treatment of several neuropsychiatric disorders. For instance, the action of AEA binding at CB1 and of PEA at PPAR-α has been associated with a fast improvement of emotional behavioral deficits, including aggressive behavior and impulsivity (11, 12), which are behavioral endophenotypes of human behavioral-traits of suicide risk. In humans, studies show higher CB1 and CB1-mediated G-protein activation in the dorsolateral prefrontal cortex (DLPFC) of suicide victims (13). Studies conducted in alcoholic suicide victims have evidenced enhanced CB1 activation and increased AEA and 2-AG concentrations in the DLPFC (14). Furthermore, CB1 expression was increased in the ventral striatum of suicide individuals who struggled with alcoholism (15). Intriguingly, both FAAH expression and activity was found upregulated in post-mortem brain of suicide subjects (15). Together, these findings underlie profound deficits within the endocannabinoid system. More studies are warranted to understand the precise role of endocannabinoid levels, their biosynthetic enzymes as well as their receptors (CB1 and PPAR-α) in suicide victims.

the analyses, AEA and PEA serum concentrations still remained elevated. This study supports a role for AEA and PEA in the pathophysiology of suicidal behavior. However, this limited study should be expanded and replicated in larger cohorts (42).

In preclinical studies, deletion of the gene encoding CB1 induces aggressive behavior in male mice following the exposure of a same-sex conspecific "intruder" in their home cage –a behavioral trait of suicide-like behavior (43). Interestingly, a later study conducted by the same group shows the relevance of CB2 receptors in the development of the suicidal-like phenotype in mice. CB2-KO mice present higher levels of aggressive behavior both in the social interaction and the resident intruder paradigms compared to wild-type mice (43).

The content of PEA was found altered in several diseases and disorders, which include multiple sclerosis, traumatic brain injury, chronic pain, neuroinflammation, and various neurodegenerative diseases (23). Notwithstanding its role and that of its congeners remains largely underinvestigated in psychiatric disorders, recent studies observed that PEA, OEA, and stearoylethanolamide (SEA) levels are significantly reduced in male and female patients in a manner that correlated with severity of PTSD symptoms (44). This finding is in line with preclinical studies that have showed that PEA concentrations were elevated following antidepressant treatment in corticolimbic areas of rodents (45) and that administration of PEA improves fear extinction and anxiety-like behaviors, a pharmacological action that is abolished in PPAR-α-KO mice or after administration with PPAR-α antagonists (11). In depressed patients, PEA increases the pharmacological efficacy of the antidepressant citalopram in improving depressive symptoms (46). These observations are further supported by studies showing that physical exercise exert a strong antidepressant effect and this action correlated with enhancement of AEA, PEA, and OEA levels in PTSD and MDD subjects (47).

THE POTENTIAL ROLE OF THE ENDOCANNABINOID SYSTEM AS A BIOMARKER OF MOOD DISORDERS AND SUICIDE

The endocannabinoid system is a neuromodulatory system among the most expressed in human and rodent brain and implicates the action of several other neurotransmitter systems, including the GABAergic, glutamatergic, and serotonergic, for the most part. Its role in the regulation of emotions has significantly advanced our understanding of the pathophysiological mechanisms leading to mood disorders. Developing reliable biomarkers for mood disorders remains one urgent goal in molecular psychiatry so that patients at risk can be timely protected by highly debilitating conditions, such as major unipolar depression and PTSD that are highly comorbid with suicide. This relies on establishing animal models that closely mirror these prevalent stress-induced pathological conditions and establishing sophisticated technology to achieve this goal.

The summary above substantiates the concept that the endocannabinoid system is a novel and potential target underlying the neurobiology of mood disorders and suicide and may serve to exploit new treatments. Indeed, both preclinical and clinical studies show that the CB1 receptor and the endocannabinoids, AEA and 2-AG may play a role in suicide behaviors. Recent studies also suggest a role for the PPAR-α receptor and its endogenous modulators, PEA, OEA and SEA in PTSD and depression and in aggressive behavior and impulsivity in animal models of these mood disorders (12, 44). A better characterization of these systems would benefit the field of neuropsychopharmacology to better comprehend the endocannabinoid role in the mechanisms of mood disorders and suicide pathophysiology. This will also facilitate designing more efficacious preventive strategies to anticipate suicidal attempts.

Suicide is a rather complex psychiatric disorder that remains poorly understood and likely involving several neurotransmission systems, neuropeptides and neurohormones in addition to the role played by the endocannabinoid system. Evidence shows that PPAR-α engages the biosynthesis of the GABAergic neurosteroid, allopregnanolone to modulate emotional behavior, including fear responses and aggressive behavior (11, 12). Importantly, allopregnanolone is implicated in the pathophysiology of PTSD and depression and the US FDA has recently approved it as the first specific treatment for the treatment of post-partum depression (48). Hence, investigation on the neuronal circuitry and functional crosstalk between the endocannabinoid system and the neurosteroid biosynthesis may unveil more precise neurobiological targets underlying mood disorders and comorbid suicidal behaviors that may prove essential in developing novel therapeutic target for the treatment of these conditions.

New knowledge on the role of the endocannabinoid system in human pathophysiology has been allowed by quantifying serum/plasma endocannabinoids in patients with several neuropsychiatric conditions by gold standard technology. Some studies have explored endocannabinoids and related N-ethanolamines in saliva and studied how they change in relation to various pathophysiological conditions. For example,

fasting plasma and salivary levels of endocannabinoids were quantified through liquid chromatography-mass spectrometry (LC-MS). While no studies have investigated the levels of the endocannabinoids, 2-AG and AEA, and their congeners OEA and PEA in blood vs. saliva in psychiatric disorders, these endocannabinoids were reliably quantifiable in saliva obtained by obese subjects. Their levels were significantly higher in obese than in normal subjects suggesting that salivary endocannabinoid levels might represent a useful biomarker in obesity (49). Novel investigations should address whether endocannabinoid levels assayed by state-of-the-art technology, including GC-MS or LC-MS, that provide unsurpassed structure selectivity and sensitivity, may correlate in blood and saliva and whether they also predict severity of psychiatric symptoms.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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Cannabidiol and Neurodevelopmental Disorders in Children

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Neurodevelopmental and neuropsychiatric disorders (such as autism spectrum disorder) have broad health implications for children, with no definitive cure for the vast majority of them. However, recently medicinal cannabis has been successfully trialled as a treatment to manage many of the patients' symptoms and improve quality of life. The cannabinoid cannabidiol, in particular, has been reported to be safe and well-tolerated with a plethora of anticonvulsant, anxiolytic and anti-inflammatory properties. Lately, the current consensus is that the endocannabinoid system is a crucial factor in neural development and health; research has found evidence that there are a multitude of signalling pathways involving neurotransmitters and the endocannabinoid system by which cannabinoids could potentially exert their therapeutic effects. A better understanding of the cannabinoids' mechanisms of action should lead to improved treatments for neurodevelopmental disorders.

Keywords: anxiety, autism, cannabinoid, cannabidiol, endocannabinoid system, neuroinflammation, neuropsychiatry, paediatrics

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NEURODEVELOPMENTAL AND PSYCHIATRIC DISORDERS

Neurodevelopmental disorders in children have profound impacts on the functioning of children and families particularly where an additional mental health diagnosis is present. The prevalence of any neurodevelopmental disorder seems to vary depending on the study; however, it seems to be around 15% of children (3–17 years) in the United States of America (USA) based on parental concerns (1). This includes diagnoses such as Attention Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), Intellectual Disabilities (ID) and syndromic disabilities. According to Boyle et al., around 4% of affected children had at least 2 diagnoses. Those who have a neurodevelopmental disorder are often two to four times more at risk of developing a mental health problem than a typically developing child (1). Neurodevelopmental disorders can include anxiety and mood disorders, Tourette's syndrome, psychosis, and bipolar disorders. Individuals with neuroatypical presentations may pose particular challenges to assessment and understanding of the psychiatric diagnosis. They may resort to behavioural escalations (such as tantrums and self-injury) as a manifestation of their extreme distress and inability to communicate their distress and, as such, can be very difficult for families and communities to support (2, 3).

The aetiology of neurodevelopmental disorders is multifactorial with polygenic risk as well as the impact of perinatal exposures to biological or environmental factors that may act as

epigenetic modifiers of neuronal networks and structures. The biological underpinning of many of these disorders is only, in part, minimally understood and thus therapies are usually based on responses in typically developing individuals, older paediatric populations and adults. Treatment options for comorbid mental health problems are limited on the whole to symptomatic therapies and often evidence is restricted in these populations as to the treatment's effectiveness and the mechanisms involved. For example, stimulants for ADHD and some newer therapies are frequently used where attention and impulsivity issues are present in other, non-ADHD disorders while anxiety medication may be trialled off-label on a child with ID diagnosed with significant anxiety. Interestingly, a common trait of ASD and ASD-related disorders (such as Fragile X syndrome and 22q11.2 deletion syndrome) is anxiety and seizures (with or without epilepsy) (4–7). The use of atypical antipsychotics continues to be one of the only evidence-based treatments in children with autism and escalated behaviour; however, the side effect profile of antipsychotics is very difficult to manage, which relegates them to be used only as a short-term last resort. Clinicians are regularly trialling medication to support children and families in significant distress, leading to most of these medications to be prescribed off-label for neuroatypical children; therefore, new medications with clear relationships to aetiology and biological underpinnings are required to support these individuals as they develop into adulthood.

CANNABINOIDS AS POTENTIALLY THERAPEUTIC FOR PAEDIATRIC PSYCHIATRIC DISORDERS

There has been interest for a long time in the impact of medicinal cannabis on neurological and psychiatric disorders (8). Phytocannabinoids (cannabinoids) have been found to be molecules that could be pharmaceutically beneficial for some ailments (9). However, the prescription of medical cannabis has been very conservative because of its stigma as a substance of abuse in many jurisdictions (10). Thanks to some well-publicised case studies, a recent increase in community acceptance of cannabis's medical benefits (11) has been shifting government policy in favour of cannabis decriminalisation/legalisation in jurisdictions such as Canada, Israel, Uruguay, a majority of USA states, and the Food and Drug Administration (12–15). In Australia, the Therapeutic Goods Administration (TGA) currently allows strict, limited prescription of medical cannabis by registered medical practitioners (16), and in 2019 the Australian Capital Territory legalised the individual possession and cultivation of small amounts of cannabis (17). Consequently, this surge in therapeutic cannabinoid usage is encouraging a rise in cannabis research, as the cannabis farming industry, biotechnology and pharmaceutical corporations compete to develop more medical cannabinoid products and better commercialise their usage.

Among the 126 cannabinoids in the cannabis plant and its many variants (18), only delta-9-tetrahydrocannabinol (Δ9-THC or THC) is strongly psychoactive and its effects on the developing brain have been a concern for many clinicians as it can induce short-term alterations in mood, behaviour, appetite and cognition (19). Pathological and behavioural aberrations have been detected in chronic cannabis users and can vary with individuals as well as over time (20, 21), making the effects of long-term cannabis treatment on individuals difficult to predict with current methodology. The neurodevelopment of children and adolescents can be disrupted by the cannabinoids' wide-ranging effects on the central nervous system (CNS) (22). The uncertainty of THC's long-term safety has directed society's contemporary focus on cannabidiol (CBD) as the most promising therapeutic cannabinoid due to its relative abundance in the plant, lack of psychoactive effects, positive safety profile (23) and purported benefits (24). There are some synergies between THC and CBD [i.e., THC can reinforce CBD's beneficial properties while CBD dampens THC's psychotropic effects (25, 26)], but THC's psychoactive properties and strong neural interactions can be detrimental after long-term frequent exposure, especially in the developing brain. Indeed, significant alterations in brain structure/function have been observed in humans, adult and adolescent rodents (27–31) frequently consuming cannabis compared to cannabis-free controls. But there is no definitive consensus as other experiments have either reported no significant difference in brain morphology (32) or have been contradictory; for example, one study found thinner brain cortices in adolescent/young adult cannabis users (33) while another study reported increased cortical thickness in adolescent cannabis users (34), compared to non-users of cannabis. Such uncertainty about the long-term effects of cannabinoids on the human brain reinforces the need for in-depth investigations of the cannabinoids' positive and negative effects. There is still very little understanding of how the intake of THC, CBD, and/or other cannabinoids may affect developing neurodivergent brains and research is urgently needed as the use of medicinal cannabis becomes legalised in various parts of the world.

The precise mechanisms behind CBD's beneficial effects are currently not well-understood. CBD does not significantly interact with the cannabinoid receptors that THC interacts strongly with, and its actions have been attributed to inhibition of anandamide degradation (35), serotoninergic, anti-inflammatory and/or its antioxidant properties (36–39).

Abbreviations: 22QS, 22q11. 2 deletion syndrome; 2-AG, 2-arachidonoylglycerol; 5-HTR, 5-hydroxytryptamine receptor; AA, Arachidonic acid; ADHD, Attention Deficit Hyperactivity Disorder; AEA, N-arachidonoyl-ethanolamine or anandamide; AEDs, Anti-epileptic drugs; ASD, Autism Spectrum Disorder; BBB, Blood-brain barrier; CBD, Cannabidiol; CB1R, Cannabinoid receptor 1; CB2R, Cannabinoid receptor 2; Cys-LT, Cysteinyl leukotriene; CNS, Central nervous system; COX, Cyclooxygenase; CYP, Cytochrome P450; EA, Ethanolamide; ECS, Endocannabinoid system; EET, Epoxyeicosatrienoic acid; ENT1, Equilibrative nucleoside transporter; FAAH, Fatty acid amide hydrolase; FDA, Food and Drug Administration; FXS, Fragile X syndrome; GABA, γ-Aminobutyric acid; GPR, G-protein coupled receptor; HETE, Hydroxyeicosatetraenoic acid; HPETE, 5-hydroperoxyeicosatetraenoic acid; ID, Intellectual disabilities; IL-1β, Interleukin-1β; LOX, Lipoxygenase; LT, Leukotriene; MAGL, Monoacylglycerol lipase; MAM, Methylazoxymethanol acetate; PG, Prostaglandin; TGA, Therapeutic Goods Administration; TNF-α, Tumour necrosis factor-α; Δ9-THC or THC, Delta-9 tetrahydrocannabinol; TRPV, Transient Receptor Potential Vanilloid; VDAC1, Voltage-dependent anion selective channel protein 1.

Therapeutic administration of CBD has been demonstrated to alleviate a range of neuropsychiatric symptoms in schizophrenia (35, 40, 41), depression (42) and anxiety (24, 43, 44) (**Table 1** summarises a selection of experiments/trials). Encouraged by these findings, CBD therapy has recently been clinically tested in case studies of autism. Aran (3) and Barchel et al. (46) reported improvements in behaviour, anxiety, and communication in oral CBD treatment trials with ASD children—about 60–70% of patients responding well to the treatment, with the side-effects of somnolence and appetite loss being reasonably tolerated. Phase 1b-2 trials of CBD therapy in ASD have demonstrated a positive response in irritability scales on the Aberrant Behaviour Checklist-Community (ABC-C) as well as some core features such as hyperactivity, anxiety. Other trials in phase 2 and phase 3 are underway for anxiety/ behavioural outcomes in ASD, 22q11.2 deletion syndrome (22QS), ID and Tourette's syndrome, with the results of phase 3 studies being awaited. In the case of Fragile X syndrome (FXS) treatment, positive results have also been obtained with successful case studies (5) and clinical trials (45) that involved the participation of children; the studies reported clinically significant improvements in emotional and behavioural symptoms of FXS, namely anxiety, social avoidance, and irritability. The CBD in Heussler's study was administered by transdermal application of a CBD gel patented by Zynerba Pharmaceuticals (4). Most side-effects were mild enough for this novel CBD treatment to be deemed tolerable by the FXS patients (45). Unlike ASD and FXS, there have been no reports published on the efficacy of CBD treatment on 22QS patients as of the time of writing. There is an ongoing clinical trial sponsored by Zynerba Pharmaceuticals, where the efficacy of their CBD gel is being tested on 22QS minors. Due to the commonalities shared by ASD, FXS, and 22QS, the rationale is that CBD would exert anxiolytic and behavioural improvements, resembling those observed in CBD therapy of ASD and FXS (4, 45).

With many cases of epilepsy persistently resistant to the most common treatment options (48), families of affected epileptic individuals have advocated for the use of medical cannabis as an alternative treatment. CBD demonstrably acts on brain regions and neural pathways in animal and human models of epilepsy via anticonvulsant and neuroprotective effects (38, 49–52). Therefore, cannabinoids (particularly CBD) have been trialled for the management of epilepsy. Paediatric clinical trials are underway in many parts of the world to evaluate pharmaceutical CBD and its impact on a number of areas including completed randomised clinical trials in Dravet and Lennox-Gastaut syndromes (refractory epilepsy syndromes). Two trials focused on the treatment of Lennox-Gastaut syndrome while one trial selected patients affected by Dravet syndrome. All trials had participants regularly administered with a patented oral formulation of 98% CBD (Epidiolex® by GW Pharmaceuticals). In these trials, the participants' pre-existing treatment regime (including medications and/or interventions for epilepsy, such as a ketogenic diet and vagus nerve stimulation) remained unchanged throughout. According to these trials' findings (47, 53, 54), CBD-based pharmaceutical formulations show promise as effective supplementary anticonvulsants, especially to treat refractory epilepsy (55, 56).

Cannabinoid researchers are still attempting to determine the precise effects of each cannabinoid on the human body, and their interactions with each other as well as other xenobiotics (25). Challenges in developing the evidence base for clinical prescribing have been related to products of variable quality with minimal understanding of how various cannabinoids work either individually, together (entourage effect) or with other drugs. One of the ways by which the cannabinoids have been demonstrated to exert their effects is by their direct and indirect interactions with a crucial component of the CNS, called the endocannabinoid system (ECS) (57–59). The ECS is intrinsically linked to neuromodulation, and therefore may be critical in alleviating some neuropsychiatric symptoms (44, 60).

A BRIEF INTRODUCTION TO THE ENDOCANNABINOID SYSTEM

The ECS is a major axis of the CNS, primarily responsible for modulating excitatory and inhibitory synaptic activity through the release of endogenous cannabinoids (endocannabinoids) that interacts with cannabinoid (and non-cannabinoid) receptors (61). Critical features of neural development/health and synaptic plasticity are regulated by the ECS (62). The lipid-based endocannabinoids are secreted extracellularly from the post- to the pre-synaptic site where they bind to cannabinoid receptors to initiate retrograde synaptic signalling (i.e., a negative feedback mechanism that regulates pre-synaptic activity) (63). The cannabinoid receptors, belonging to the G-protein coupled receptor (GPR) family, are found throughout the entire human body—the most well-characterised receptors being the Cannabinoid 1, Cannabinoid 2 and GPR55 receptors.

Cannabinoid 1 receptors (CB1Rs) are particularly abundant in the basal ganglial, cerebellar, cortical and hippocampal regions, with the majority of them present on axon terminals and pre-terminal axon segments (61, 64). CB2 receptors (CB2Rs) are normally expressed at much lower levels in the CNS compared to CB1Rs; this receptor is primarily present in microglia, vascular elements, immune cells and some specific neurons (61, 65). However, when the blood-brain barrier (BBB) is disrupted (by insults such as neuroinflammation), CB2R expression levels in the brain increase due to immune cells flooding the CNS (66). The majority of GPR55 receptors are aggregated in the CNS and peripheral nervous system (67, 68), where their activation on neurons can upregulate intracellular calcium release and inhibit potassium release, resulting in increased neuronal excitability (69, 70).

Activation of the cannabinoid receptors by endocannabinoids can trigger downstream signalling, such as ion channel openings, changes in intracellular calcium ion concentrations and regulation of inflammatory pathways (71). The two most well-studied endocannabinoids are N-arachidonoyl-ethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). AEA acts as a high-affinity, partial agonist of CB1R, and barely interacts with CB2R while 2-AG is a full agonist at both CBRs with low-to-moderate affinity, with both endocannabinoids being GPR55 agonists (68, 72, 73). At the end of their normal

TABLE 1 | Summarised findings of some referenced experiments/clinical trials in humans, which demonstrate the wide range of neurological disorders that CBD therapy could potentially be effective for.

References	Disorder	Experimental/clinical model	Drug dose and route	Major findings
Leweke et al. (35)	Schizophrenia	42 adult schizophrenic patients	800 mg/d, oral	Alleviation of psychotic symptoms
Heussler et al. (45)	Fragile X syndrome	20 FXS patients, aged 6–17 years	Daily 50 mg dose, twice daily 50 mg dose or twice daily 125 mg dose, transdermal	Significant reductions in anxiety and behavioural symptoms
Barchel et al. (46)	Autism Spectrum Disorder	53 children diagnosed with ASD	16 mg/kg/d (maximum of 600 mg), oral	Alleviation of some ASD comorbidity symptoms
Solowij et al. (42)	Depression	20 adult frequent cannabis users	200 mg/d, oral	Significant decrease in depressive and psychotic-like symptoms
Shannon et al. (43)	Anxiety and sleep	72 adults presenting with high anxiety or poor sleep	25 mg/d (maximum of 175 mg for 1 patient), oral	Long-term decrease in anxiety scores within the 1st month of treatment
Devinsky et al. (47)	Refractory epilepsy	120 children and young adults with Dravet syndrome and refractory seizures	5–20 mg/kg/d, oral	Reduction in convulsive-seizure frequency, but higher rates of adverse events than placebo

lifecycle, AEA is mostly degraded to arachidonic acid (AA) and ethanolamine by fatty acid amide hydrolase (FAAH) (71), while 2-AG is majorly converted to AA and glycerol by monoacylglycerol lipase (MAGL) (74). Interestingly, AEA is also a full agonist (with a different affinity than for CB1R) of a non-ECS receptor named the Transient Receptor Potential Vanilloid 1 (TRPV1) that regulates extracellular calcium ion secretion and neuronal excitability (75).

Another potential way for the ECS to affect the progression and severity of neuropsychiatric disorders is via the gut-microbiome-brain axis (76, 77). The gut-microbiome–brain axis is constituted of signalling (neural and humoral) pathways that connect the gastrointestinal system (GIS) and its microbiota to the CNS in reciprocal relationships for homeostatic and defensive maintenance of the whole body. ECS receptors, namely CB1R and TRPV1, peroxisome proliferator-activated receptor alpha (PPAR-α) and GPR119 are strongly expressed throughout the gut-brain axis (e.g., intestinal epithelial cells, myenteric and vagal fibres). These receptors affect myenteric neuron activity, vagal and sympathetic nerve function, and the release of gastrointestinal neuropeptides (such as N-acyl amides), which may subsequently have a significant impact on brain neural activity (77).

The gut microbiota produce metabolites that can interact with the ECS (78, 79). The microbes are usually categorised as either deleterious or beneficial (probiotic) to the host organism, depending on their overall effects (80). Commensal microorganism-derived molecules produce neurotransmitters (e.g., serotonin, GABA), as well as ECS-like mediators that are capable of interacting with host ECS receptors; for example, commendamide is analogous to the human signalling molecules N-acyl amides and interacts with ECS GPRs (81). Currently, the exact effects of these ligands are still mostly unknown, but their existence strongly hint at complex layers of interaction between the gut-brain axis and gut microbiota (78).

Components of the ECS can thus strongly modulate behaviour and mood via interactions with underlying neurotransmission and the gut-microbiome-brain axis.

THE ROLE OF THE ENDOCANNABINOID SYSTEM IN REGULATING ANXIETY

Anxiety is usually manifested in affected individuals as disproportionate startle response, avoidance behaviour, autonomic hyperactivity, increased muscular tension and reduced motion (66). Anxiety is primarily mediated by glutamatergic (excitatory, i.e., increase likelihood of action potentials), serotoninergic and GABAergic (inhibitory, i.e., decrease likelihood of action potentials) pathways. GABA is the main inhibitory neurotransmitter, widespread throughout the cortex and counters the excitatory activity of glutamatergic neurons (82). Excessive anxiety as experienced by patients with anxiety disorders is theorised to be caused by an imbalance between excitatory and inhibitory signalling. Consequently, such an imbalance may lead to cortical hyper-reactivity and behavioural hypersensitivity in ASD. Puts et al. (83) and Sapey-Triomphe et al. (84) found that cortical GABA levels appear to be reduced in children and adults with ASD, respectively, in comparison to those of neurotypical controls (83, 84). However, Kolodny et al. (85) recently reported no differences in cortical concentrations of GABA and glutamate between neurotypical and ASD young adults (85). This discrepancy in findings could be attributed to low participant numbers and small differences in experimental methodologies. The proper functioning of the ECS is also disrupted in FXS. The loss of Fragile X mental retardation protein (which regulates the translation and transport of messenger RNAs in brain neuron dendrites) in FXS seems to impair the glutamate receptor-5

(mGluR5)-dependent 2-AG signalling at excitatory synapses (86). Additionally, administration of AEA in a mice model of FXS (FMR1 knockout mice) reduced social anxiety (87), suggesting a detrimental downregulation of AEA in FXS.

Functional CB1Rs and CB2Rs expressed (88, 89) in GABAergic, dopaminergic, glutamatergic, and serotoninergic neurons (90–93), could be crucial in regulating behavioural and emotional states (88, 89), which are heavily disrupted in psychiatric/mood disorders. CB1Rs, in particular, are highly expressed on GABAergic interneurons (90, 94), on glutamatergic terminals (90, 92) and on dopamine D1 receptor positive neurons (95). Agonism of CB1Rs can inhibit the secretion of GABA and glutamate from presynaptic terminals (96–99), which indicate that endocannabinoid activation of CB1R can influence the type of synaptic signalling. AEA-mediated TRPV1 activation is linked to an anxiogenic response, as opposed to the anxiolytic response elicited by AEA-mediated CB1R activation. This suggests that there might be an imbalance between CB1R and TRPV1 expression that might play a part in instilling excessive anxiety (100). Inhibition of FAAH by selective inhibitor URB597 was reported to activate serotoninergic neurons in the midbrain of stressed rats, by the associated increase in AEA-mediated signalling at CB1R (101). Inhibition of FAAH and MAGL by selective inhibitors produced anxiolytic effects in CB1R-deficient mice, but not in CB2R-deficient mice, suggesting that CB2R could play a role in regulating anxiety (102). Additionally, CB2R might play a role in regulating anxiety as augmented activation of CB2R by accumulation of 2-AG (via inhibition of MAGL) was found to exert anxiolytic effects in a rat model of stress (103).

From the evidence gathered so far, therapeutic modulation of synaptic signalling and plasticity could indeed be feasible by regulation of the ECS. Moreover, a well-regulated ECS is critical in ensuring good neural health and function as distressed neural cells can lead to further neurological issues such as epilepsy (104).

HOW THE ECS COULD BE INVOLVED IN NEUROINFLAMMATION AND EPILEPSY

The ECS is an important signalling axis for inflammatory pathways throughout the body. Many children affected by ASD, FXS, and 22QS suffer from epileptic/non-epileptic seizures that stem from detrimental mutations responsible for their disorders (5, 7, 105–107). 10–30% of people with ASD have comorbid epilepsy and several synaptic plasticity pathways appear to be involved in both disorders (105). As such, affected children are at increased risk of serious seizure-related accidents and have their neurodevelopment further impaired by frequent seizures (108). In recent years, epilepsy has been surmised to be strongly correlated with neuroinflammation (104, 109). Additionally, abnormally high levels of neuroinflammation have been associated with ASD (110); Vargas et al. (110) and Jyonouchi et al. (111) found higher levels of proinflammatory cytokines (e.g., tumour growth factor–β1) in the brain tissue, cerebrospinal fluid and peripheral blood of ASD patients (including children) (110, 111).

Neuroinflammation is the term given to a set of defensive responses to insult and/or injury in the neural environment that is mainly mediated by glial cells. The resident immune cells of the CNS, the microglia, primarily function in protecting the neuronal population; they are called into action by inflammatory stimuli such as foreign bodies, products from injured/inflamed neurons, blood-brain barrier disruptions, and by chemokines/cytokines [e.g., Interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α)] (112–114). Neuroinflammation is a protective physiological process but can be harmful when it is excessive and unregulated (115). Multiple parts of the ECS are involved in inflammatory pathways. Moreover, microglia express many components of the ECS (such as CB1R, CB2R and GPR55), via which they communicate with neurons via expression of endocannabinoids (116, 117). There is evidence of microglial involvement in ASD from both brain tissue immunohistochemistry and positron-emission tomography (PET)-imaging studies which revealed increased neuroinflammation and population of activated microglia in brains of ASD patients (118, 119) compared to non-ASD individuals. Therefore, artificially modulating microglial endocannabinoid signalling and treating neuroinflammation could potentially alleviate some ASDsymptoms (117).

Agonism of CB1R and CB2R have shown anti-inflammatory effects in human and animal models (120–123). Antagonism/non-expression of GPR55 also resulted in a reduction in neuronal and microglial inflammation (116, 124, 125). However, agonism of GPR55 in animal and human neural stem cells was found to elicit a neuroprotective effect and rescued neurogenesis after inflammatory insult (126). Additionally, activation of microglial GPR55 by the endogenous ligand l-α-lysophosphatidylinositol limited neuronal damage in rats (127). As Hill et al. suggest, the actions of GPR55 probably strongly depend on the cell type and cause of inflammation (126). Cyclooxygenase enzymes (COX) synthesise signalling intermediaries known as prostanoids, often derived from AA. The constitutive isoform of COX, COX-1, found in numerous cell types, regulates physiological responses, while the inducible isoform, COX-2, is induced rapidly in several cell types (including neurons and glial cells) after biochemical stimuli, such as cytokines and pro-inflammatory molecules (128). COX-2 is involved in the conversion of a minor proportion of AEA and 2-AG to prostaglandin ethanolamides (PG-EAs) (74) and prostaglandin glycerol esters (PG-Gs) (129), respectively—both of which can contribute to inflammatory responses (128). Other prostaglandins derived from AA by COX-1 and COX-2, prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have neurotoxic properties (125, 130, 131). Suppression of MAGL activity (which leads to a downregulation in AA synthesis) has shown neuroprotective effects in mice (132). COX-2 levels have been found to be greatly increased in the brains of patients with epilepsy, compared to non-epileptic patients (133) and in animals that experience prolonged seizures (134), suggesting a relationship between epilepsy and neuroinflammation.

The Cytochrome P450 (CYP) family is another group of enzymes that breaks down endocannabinoids. The ubiquitous CYP enzymes are expressed at different levels across the

body, with variations across species and amongst individuals. The CYP enzymes are known for their ability to metabolise xenobiotics, with the metabolites sometimes causing side-effects (135). Changes in CYP activity can influence downstream endocannabinoid signalling pathways by virtue of changes in substrate and metabolite concentrations. CYP3A4, expressed in the human brain (136, 137), derives anti-inflammatory epoxyeicosatrienoic acids (EETs) and pro-inflammatory hydroxyeicosatetraenoic acids (HETEs) from AA (138–141). AEA can be broken down by CYP enzymes (namely CYP3A4, CYP2C19, CYP2D6, and CYP2J2) into EET-ethanolamides (EET-EAs) and HETE-ethanolamides (HETE-EAs) (142–144). Just like their precursor molecules, the EET-EAs and HETE-EAs can bind to CB1Rs and CB2Rs, albeit with different affinities, e.g., 5,6-EET-EA binds much more strongly with CB2R than AEA (145) while 20-HETE-EA and 14,15-EET-EA have only a weak affinity for CB1R (146) in murine models. CYP2J2 breaks down 2-AG to create two products, 2-11,12-epoxyeicosatrienoic glycerol (EET-G), and 2-14,15-EET-G (147), which interact strongly with both CBRs (especially CB1R) (148). Many CYP metabolites therefore are potentially endogenous ligands for some of the ECS receptors and could subsequently be involved in inflammation regulation.

The lipoxygenase (LOX) enzyme pathway is another metabolic route for endocannabinoids and other related fatty acids (149). The LOX pathway starts with the change of AA into leukotriene A4 by the 5-LOX enzyme (expressed on cell types such as neurons). Leukotriene A4 (LTA4) is rapidly catalysed into LTB4 and cysteinyl leukotrienes (i.e., Cys-LTs, which comprises LTC4, LTD4 and LTE4) (149, 150). LTD4 has been linked to blood-brain barrier dysfunction (151), a contributing factor of neuroinflammation (152), as evidenced by exposure of microglial Cys-LT1 and Cys-LT2 receptors to LTD4 resulting in microglial secretion of pro-inflammatory IL-1β in mice (153). In brief, the ongoing research on eicosanoids (collective term for the endocannabinoids and the many metabolites of the ECS) indicates that the ECS is thoroughly implicated in regulation of neuronal activity and neuroinflammation. But until the signalling pathways involved are thoroughly investigated, particularly in the human brain, how neuroinflammation is exactly linked to ECS dysfunction and psychiatric impairments remains to be elucidated. Interestingly, inflammation in the GIS could substantially affect the gut-microbiome-brain axis and subsequent neuronal activity as ASD individuals have been reported to suffer from gastrointestinal issues (such as diarrhoea and constipation) (154–157) and dysbiotic microbiota compared to neurotypical individuals (158). Perturbations in gut microbial diversity has been found to influence neuroinflammation (159) as some gut microbes can secrete pro-inflammatory metabolites and cytokines (160) that cross the blood-brain barrier. CB1R, TRPV1 and PPAR-α can modulate the permeability of the gut-vascular barrier that prevents the entry of intestinal bacteria into the bloodstream; if the GVB's selective permeability is compromised, the bacteria themselves can enter the bloodstream and cross the BBB, causing an inflammatory response (161).

In summary, there is little doubt that the ECS is likely to be central in the aetiology and occurrence of neuropathology, whereby the modulation of the ECS at multiple points by extraneous agents such as cannabinoids could achieve beneficent outcomes.

CANNABINOIDS INTERACT WITH THE ECS AND NEUROTRANSMISSION

The cannabinoids' interactions with multiple receptors and enzymes can be safely assumed to hold the key to their wide-ranging therapeutical benefits, but can also obscure the exact mechanisms of their effects. THC is a partial agonist of CB1Rs and CB2Rs, and an agonist of GPR55. On the other hand, CBD's antagonistic/negative allosteric modulating actions on the CB1 and CB2 receptors (57, 162–164) might help explain how CBD can dampen THC's psychoactivity (165) (**Figure 1**).

While CBD might not interact strongly with CB1R and CB2R when administered at therapeutical levels (166), it has been reported to regulate calcium ion homeostasis in neurons (167) and increase inhibitory neurotransmission via interactions with GPR55 (164). CBD therapy has been correlated with an increase in AEA blood levels and a reduction in the psychotic symptoms of treated schizophrenic patients vs. placebo-control patients (35); the mechanism behind CBD's beneficial effect in this instance could be due to an increase in AEA levels found to be lower in the cerebrospinal fluid of epileptic patients (168) and in the blood of ASD children (169, 170). Of note, the mechanism by which CBD increases AEA levels seems to differ between species; Elmes et al. reported that, in humans, this effect may be due to CBD binding preferentially to the fatty acid binding proteins on which AEA depends to be transported into cells for FAAH catalysis rather than the CBD-induced FAAH inhibition observed in rodents (171). This interaction between CBD and AEA metabolism in humans vs. rodents (171, 172), highlights that the differences in xenobiotics metabolism between species can limit the utility of animal models in cannabinoid research.

In animal models of ASD, an increase in AEA concentration has been correlated with improvements in social interactions. AEA can interact with oxytocin, a neuropeptide that promotes parental and social bonding. Indeed, recent evidence has demonstrated that oxytocin stimulates AEA release in the nucleus accumbens, a key region for the reinforcing properties of natural rewards, with AEA-mediated signalling a requirement for the pro-social effects of this neuropeptide (173). A model of defective oxytocin-driven AEA signalling in ASD could therefore explain how CBD intake ameliorates social interactions in ASD patients (3, 46). Upregulated *Magl* gene (gene that encodes for the MAGL enzyme) expression has been observed in rat hypothalami treated with 10 mg/kg THC (174), supporting the hypothesis that cannabinoids can modulate cerebral endocannabinoid tone. Cannabinoids, like CBD, have been found to inhibit COX-2 activity and hence reduce the production of pro-inflammatory prostaglandins, which could be an additional pathway by which cannabinoids increase the levels of the endocannabinoids, triggering an indirect anti-inflammatory and

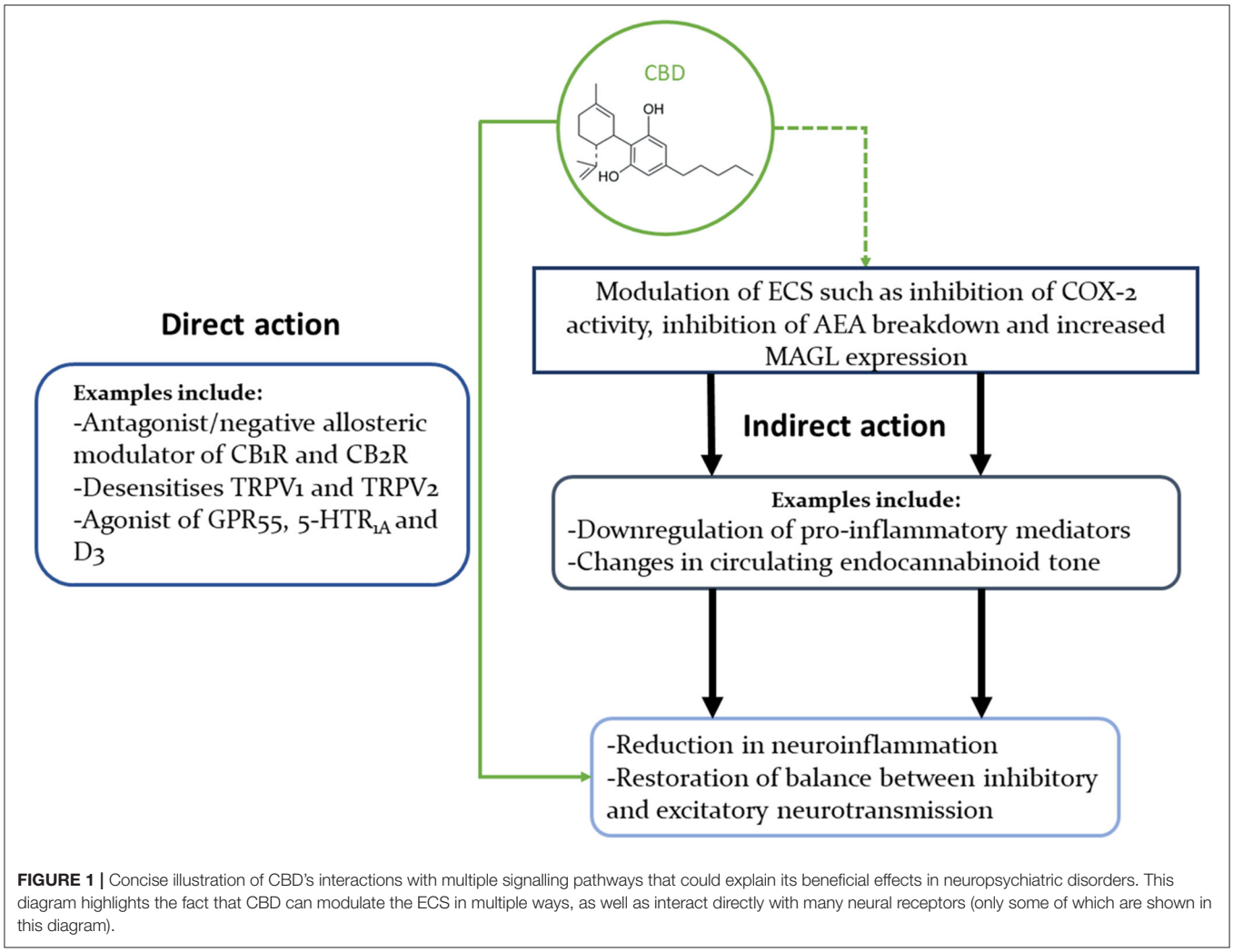

FIGURE 1 | Concise illustration of CBD's interactions with multiple signalling pathways that could explain its beneficial effects in neuropsychiatric disorders. This diagram highlights the fact that CBD can modulate the ECS in multiple ways, as well as interact directly with many neural receptors (only some of which are shown in this diagram).

anti-epileptic activity (175, 176). CBD's inhibition of cerebral CYP isoenzymes could, in turn, modulate the levels of EETs, EET-EAs and HETE-EAs. Therefore, even though CBD may not have a high affinity for CB1R, CB2R, and GPR55, the activation of these endocannabinoid receptors may be indirectly affected by CBD's upregulation/downregulation of endocannabinoids and eicosanoids (136); for example, Bornheim et al. found that CBD inhibited the CYP-driven formation of some AEA metabolites in mice (177) while Arnold et al. reported that THC and CBD inhibited the production of EET-EAs by cardiac CYP2J2 (178). Additionally, the activity and metabolite synthesis of 5-LOX was reduced in human tumour cells treated with CBD (179). Targeted inhibition of Cys-LT synthesis significantly attenuated seizures in treated mice (compared to untreated mice) (180, 181) and in epileptic patients (182), so CBD's inhibition of 5-LOX could have an anti-inflammatory effect.

Intriguingly, CBD has been shown to desensitise non-cannabinoid TRPV1s (75) and related TRPV2s, hence blocking the release of calcium ions outside cells and dampening hyperexcitability (contributor to aberrant neuronal activity) in neurons, suggesting another potential regulatory mechanism (172, 183). CBD has been reported to enhance microglial phagocytosis in rodent microglia partially via the activation of TRPV1 and probably TRPV2 receptor channel of the microglial cells (112); however, Hassan et al. cautioned that increasing microglial phagocytosis might not be a positive strategy for combating neuroinflammation, but their results might not be applicable to human physiology.

As we highlighted beforehand, the cannabinoids may indeed exert their effects differently between species. Another case of CBD's promiscuous interactions is its agonistic actions on the serotonin (5-hydroxytryptamine-1A) receptors (5-HTR_{1A}), which are deeply involved in activating anxiolytic responses and in neuronal electrochemical activity (36, 184, 185). In healthy and ASD human adults, CBD suppressed the activity of excitatory glutamatergic neurons in the prefrontal cortex via activation of 5-HTR_{1A} (186), which could contribute to restoring the balance between inhibitory and excitatory neurotransmission. Additionally, CBD inhibits the equilibrative nucleoside transporter (ENT1) responsible for the synaptic uptake of

adenosine, thereby increasing levels of extracellular adenosine. Consequently, an upregulation in extracellular adenosine can cascade into a decrease in neuronal hyperexcitability (187–189). CBD has anti-oxidative and anti-inflammatory properties that could counter neuroinflammation; modulation of TRPV1, CB2R, and GPR55 receptors can lead to downregulation of enzymes involved in the production of pro-inflammatory PGs, reactive oxygen species, and cytokines (190, 191). Another potential avenue for CBD's anti-inflammatory action could be its inhibition of voltage-dependent anion selective channel protein 1 (VDAC1) conductance, leading to a decrease in neuroinflammation (192). CBD was also found to enhance the inhibitory γ-Aminobutyric acid (GABA)'s activation of its associated $GABA_A$ receptors which regulate inhibitory neurotransmission (193) and are targeted by drugs such as clobazam; indeed, co-administration of CBD with clobazam significantly increased the inhibitory effects of GABA compared to either compound alone (194). Additionally, CBD's amplifying effects on GABA receptors could compensate for the reduced GABAergic transmission observed in FXS (195).

Lower levels of AEA (35) and higher expression/reduced methylation of *CNR1* (the gene coding for CB1R) (196, 197) in schizophrenic patients strongly suggest a pathological link with ECS dysfunction; CBD might compensate for this dysfunction by indirectly modulating endocannabinoid levels. Additionally, CBD is a partial agonist to dopamine D3 receptor, whose expression was demonstrated to be altered in the methylazoxymethanol acetate (MAM) murine neurodevelopmental model (198). Gestational MAM treatment of pregnant dams is a validated model that produces murine offspring with adult phenotype typical of schizophrenia, such as cognitive deficits, dopaminergic dysfunction, physical and behavioural abnormalities (196, 198, 199). Another murine model that mimics the development of the human schizophrenia phenotype is perinatal THC exposure of neonates as it results in similar neurodevelopmental impairments; the cognitive and social deficits were then demonstrated to be reversed by peripubertal CBD treatment (197). These experimental results reinforce the notion that early childhood treatment with CBD might be sufficient to minimise the impact of neurodevelopmental disorders into adulthood.

CBD's interactions with the GIS ECS might depend on the mode of administration; oral intake of CBD is subject to first-pass metabolism, which can result in most of the CBD being transformed by liver enzymes into its metabolites prior to reaching the gut (200). Conversely, more direct passage of CBD in circulating blood via dermal application or inhalation would hypothetically reduce CBD's availability to the GIS. Research on CBD's effects on the gut microbiome and gut ECS are few and limited to animal model studies (generally germ-free mice) (201), but CBD's anti-inflammatory properties could be potentially involved in counteracting gut cell inflammation, gut-vascular barrier leakage and subsequent neuroinflammation by dysbiotic gut microbes (202, 203).

CONCLUSION

Our review has hopefully shown that there is a strong body of evidence that early cannabinoid treatment may offer significant potential to safely alleviate many of the common symptoms affecting children with neurodevelopmental disorders. Continued research and evidence in establishing definite relationships between cannabinoid intake and alterations of the ECS are needed to determine clear risk-benefit profiles and to screen for potential individuals in whom benefit could be predicted. CBD is currently the most promising therapeutic cannabinoid for children due to its safety profile and broad-spectrum action. A fuller understanding of CBD's metabolism in the human body (especially how it might interact with the GIS and microbiota) and mechanisms of action could result in greater optimisation of cannabinoid delivery and better development of synthetic cannabinoid analogues.

AUTHOR CONTRIBUTIONS

KKC and HH contributed to the conceptualisation and writing of the article. MM the primary supervisor of KKC's research project, also reviewed, and contributed to the manuscript. All authors contributed to the article and approved the submitted version.

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From the Clinic to the Laboratory, and Back Again: Investigations on Cannabinoids and Endocannabinoid System Modulators for Treating Schizophrenia

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The present mini-review focuses on animal models of schizophrenia that have explored the effects of cannabidiol (CBD; a non-psychoactive component of cannabis) or the pharmacological manipulation of the endocannabinoid system on behavioral and cognitive outcome measures. First, results of some relevant clinical studies in this area are summarized, and then pre-clinical work on animal models of schizophrenia based on NMDA receptor antagonism or neurodevelopmental manipulations are discussed. A brief overview is given of the theoretical framework on which these models are based, along with a concise summary of results that have been obtained. Clinical results using CBD for schizophrenia seem promising and its effects in animal models of schizophrenia support its potential as a useful pharmacotherapy. Animal models have been paramount for elucidating the actions of CBD and the function of the endocannabinoid system and for identifying novel pharmacological targets, such as cannabinoid receptors and anandamide. However, more attention needs to be placed on defining and applying independent variables and outcome measures that are comparable between pre-clinical and clinical studies. The objective of this review is, on the one hand, to emphasize the potential of such models to predict clinical response to experimental drugs, and on the other hand, to highlight areas in which research on such models could be improved.

Keywords: schizophrenia, animal model, cannabidiol, endocannabinoid, anandamide

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INTRODUCTION: FROM ANECDOTE TO CLINICAL TRIALS AND ANIMAL MODELS

During the 1940s−50s, the principle bioactive components of cannabis–cannabinoids–were identified as delta-9 tetrahydrocannabinol (THC; the psychoactive component) and cannabidiol (CBD), through the work of Adams et al. in the USA, and Todd et al. in Great Britain [reviewed in (1)]. In the 1980s−90s, the discovery of endogenous cannabinoid receptors in the nervous system (2, 3) and their endogenous ligands anandamide and 2-arachidonylglycerol (2-AG) (4, 5) provided a physiological framework within which to investigate the psychoactive properties of THC, the possible therapeutic value of cannabinoids, and the function of the endocannabinoid system. In the context of schizophrenia pharmacotherapy, most clinical investigations have focused on CBD.

The first published report of CBD as a possible treatment for schizophrenia was a case study of a 19-year old female patient, whose symptoms were reduced by a 4-week CBD treatment [1,200 mg/day; (6)]; however, results of later case studies were equivocal (7). More recently, studies showing positive results include a double-blind randomized clinical trial comparing CBD (20 patients) to amisulpride [19 patients; (8)]. Dosing of CBD began at 200 mg/day, was raised to 800 mg/day during the first week and was maintained at that level for 3 weeks. CBD was equally as effective as amisulpride, both showing significant reductions in PANSS positive, negative, and total scores. In CBD treated patients, changes in PANSS total scores were negatively associated with increases in serum anandamide, suggesting that therapeutic effects of CBD were related to an inhibition of anandamide degredation. More recent studies indicate that CBD blocks human fatty acid amide hydrolase binding proteins (FABPs), thereby preventing transport of anandamide to fatty acid amide hydrolase (FAAH, the enzyme that degrades anandamide) (9). A second study was a randomized, double-blind, placebo controlled study comparing CBD (1,000 mg/day; 42 patients) to placebo (40 patients) as an add-on to their ongoing antipsychotic treatment (10). CBD reduced PANSS positive, but not negative or total, symptom scores. Executive function, assessed by the Brief Assessment of Cognition in Schizophrenia (BACS), showed a nearly significant improvement in the CBD group ($p = 0.068$). By contrast, a double-blind, placebo-controlled study of CBD (600 mg/day) as an add-on to ongoing antipsychotic found no effect of CBD on PANSS scores or on cognitive symptoms (11). Apart from CBD dosing, these two studies differed in ethnic composition, being predominantly European Caucasian in the McGuire study vs. a more mixed race population in the Boggs study. Additionally, there could have been important differences in CBD-antipsychotic interactions, if the two patient populations differed with respect to specific antipsychotics used. Finally, responsiveness of positive symptoms to CBD in the McGuire study was quite modest, and while CBD reduced positive symptoms similarly in the Boggs study, there was a significant placebo effect. Three randomized, double-blind, placebo controlled studies tested rimonabant [a cannabinoid receptor type 1 (CB1) inverse agonist]. Two of these studies, one testing rimonabant alone [20 mg/day; (12)] and the other as an add-on to ongoing antipsychotic treatment in overweight patients [20 mg/day; (13)] found no significant effects of rimonabant on positive or negative (12), or cognitive (13) symptoms. A third randomized pilot study (14) in a small group of overweight patients reported that rimonabant (20 mg/day) had no effect on negative symptoms, but improved anxiety and hostility subscales of the Brief Psychiatric Rating Scale (14).

EXPERIMENTAL PARADIGMS FOR MODELING SCHIZOPHRENIA IN LABORATORY RODENTS: INDEPENDENT AND DEPENDENT VARIABLES

Although the "classical" criteria of face, predictive, and construct validity are those most often applied for evaluating animal models of neuropsychiatric disorders (15), another useful context for evaluating an animal model is to consider it in terms of an experimental paradigm having a set of independent and dependent variables (16–18). Independent variables include species and sex of the subjects, their genetic characteristics, and the experimental manipulation applied. Dependent variables include quantifiable neurobiological or behavioral endpoints. An assessment of the model would first critically consider whether the model's independent and dependent variables are homologous to known pathogenic risk factors and psychiatric symptoms, respectively. A second consideration would be whether the relationship between the independent and dependent variables corresponds to the real-life relationship between risk factor and psychiatric symptoms ("inductive validity") (17). Regardless of the specific criteria of validity that are applied, such criteria should be considered as a means to define the strengths and limitations of the model in order to provide a context within which to critically interpret the results that the model generates.

In addition to (and often distinct from) modeling the pathophysiology of a psychiatric disorder, an important *practical* function of an animal model is to accurately predict pharmacological responsiveness in the clinic, or "predictive validity" (15). This mini-review will focus on pre-clinical studies that have investigated CBD or pharmacological manipulations of the endocannabinoid system in animal models of schizophrenia, with the following objectives: (1) determine how well animal models of schizophrenia predicted or corresponded to clinical responsiveness, and (2) evaluate these same animal models within the context of inductive validity, as defined above.

Behavioral Outcome Measures (Dependent Variable)

Behavioral outcome measures that have been considered in studies of CBD or pharmacological manipulation of the endocannabinoid as possible treatments for schizophrenia include pre-pulse inhibition (PPI), tests of cognition, and tests of social deficits.

Pre-pulse Inhibition

Operationally, PPI is the capacity of a non-startling auditory pre-stimulus to inhibit the startle response to a startling auditory stimulus delivered 30–500 ms later. People with schizophrenia, as well as their first-degree relatives, show a reduction in this measure (19). Reduced PPI is an indication of deficient sensorimotor gating, and can be easily measured in both humans and in non-human animals.

Cognition

The MATRICS initiative (Measurement and Treatment to Improve Cognition in Schizophrenia) launched by the National Institute of Mental Health (NIMH) defined seven cognitive domains that are disrupted in schizophenia: working memory, visual learning and memory, verbal learning and memory, processing speed, attention and vigilance, reasoning and problem solving, and social cognition (20, 21). A complementary initiative, the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) identified translational cognitive tasks with construct validity that can

be applied in humans and in rodent models (22). These tasks encompass cognitive domains of attention, cognitive control, declarative memory, reward learning, action selection/preference based decision making (23).

In practice, one of the most frequently applied cognitive tests in animal models of schizophrenia is the novel object recognition (NOR) test, which is considered a test of episodic memory (24). In general, this test comprises a habituation phase, in which the rodent is allowed to explore two objects. After a brief inter-trial interval, the animal is exposed to one of the original objects along with a novel object. The unconditioned response of a rodent in these circumstances is to explore the novel object; exploration of the novel object relative to the familiar is taken as a measure of object recognition memory. This test has many practical advantages, but its relationship to the specific cognitive domains that are compromised in schizophrenia is unclear.

Social Deficits

People with schizophrenia show deficits in social cognition, as well as negative schizotypy. Schizophrenia is associated with deficits in social perception, theory of mind (understanding others' mental states), emotion perception (understanding social cues), emotion processing, and social knowledge (25). General cognitive deficits accounted for a substantial portion of the variance in social perception and emotion perception, but social cognition *per se*, independently of cognitive deficits, contributed significantly to overall social function (25, 26). Social deficits were mildly correlated with negative symptoms and a lesser extent with positive symptoms (27). Social anhedonia, a core aspect of negative schizotypy, refers to reduced motivation and reward associated with social interactions. The relationship between negative schizotypy and social cognition is unclear; nevertheless, social anhedonia and impaired social functioning in general are clear risk factors for schizophrenia (28).

Alterations in social cognition domains are challenging to measure in rodents. Two basic behavioral paradigms have been employed in this regard: the Social Interaction (SI) and social recognition (SR) tests (29). In the SI test, 2 individuals that had received the same experimental treatment are placed inside an open field arena, and the number of social interactions is the principal outcome measure. The SR test is analogous to the NOR test described above, except the test stimuli are conspecifics, rather than objects. While reduced social interaction in the SI test could be interpreted as social anhedonia, it is perhaps more difficult to relate performance deficits in the SR test to specific any specific social cognition domain.

Controlled Experimental Manipulations (Independent Variable)

Ideally, the experimental manipulation applied for inducing neurobehavioral pathology in an animal model should correspond to a known disease risk or pathogenic factor(s), and should be relatable to an existing theoretical framework for pathogenesis. The present review will focus on studies of CBD or endocannabinoid system modulation in three schizophrenia models: acute and subchronic challenge with an NMDA receptor antagonist, and neurodevelopmental models. Results of these studies are summarized in **Table 1**.

EXPERIMENTAL MODULATION OF THE ENDOCANNABINOID SYSTEM IN NMDA RECEPTOR ANTAGONIST AND NEURODEVELOPMENTAL MODELS OF SCHIZOPHRENIA

Based on reported similarities between the psychotropic effects of PCP and ketamine (glutamate NMDA receptor antagonists) and the positive, negative, and cognitive symptoms of schizophrenia, it was proposed that schizophrenia may be associated with a generalized hypofunction of NMDA receptors (51). This generalized dysfunction is believed to alter cortical information processing by altering the firing of cortical GABAergic interneurons, as well as disrupt the balance of cortical—subcortical dopamine (52). Considering this theoretical framework for schizophrenia pathophysiology, two general approaches have been taken for modeling schizophrenia in rodents: acute or subchronic/chronic challenge with NMDA receptor antagonist.

Acute Challenge With NMDA Receptor Antagonist

Three different non-competitive NMDA receptor antagonists have been used in rodent models of schizophrenia: ketamine, phencyclidine (PCP), and MK-801. Immediate effects of NMDA receptor antagonism in human subjects are similar to acute psychosis, and experimental results derived from this model are best considered within that context. Following is a summary of results of studies of endocannabinoid system manipulation in rodent models of schizophrenia based on acute challenge with NMDA receptor antagonist, considering the outcome measures of PPI, cognitive tests, and social interaction tests.

PPI

In a study of male Swiss mice, 0.3 mg/kg MK-801 significantly increased the startle response and reduced PPI, and this effect was prevented by CBD (5 mg/kg). The therapeutic effect of CBD was blocked by capsazepine (a TRPV1 receptor antagonist) (30). In another recent study of male Swiss mice, PPI alterations induced by MK-801 were not prevented by rimonabant or by WIN 55,212-2 [full agonist at the cannabinoid receptor type 2 (CB2)] (34). Contrary to results obtained in male Swiss mice, in male Sprague Dawley rats CBD (3–30 mg/kg) did not prevent MK-801-induced PPI deficits (32), and rimonabant or AM251 (CB1 antagonist) prevented PPI deficits induced by MK-801 and PCP (33).

Cognitive Deficits

In male Swiss mice, MK-801 induced deficits in memory acquisition, consolidation, and retrieval in the passive avoidance task that were prevented by AM 251, but not by oleamide (CB1 agonist) (44). Inhibitors of enzymes that degrade anandamide or 2-arachidonoylglycerol (2-AG), respectively, reduced and

TABLE 1 | Summary of studies on the effects of CBD or endocannabinoid system modulators on deficits in PPI, cognition, or social interaction in animal models of schizophrenia.

Outcome measure	CBD	CB1/CB2	Anandamide/2-AG	TRPV1	5-HT1A
PPI	1. **Prevention;** *Acute NMDA, mice;* (30) 2. **No effect;** *SC/C NMDA, mice;* (31) 3. **No effect;** *Acute NMDA, rat;* (32)	1. **Prevention;** Rimonabant, AM251; *Acute NMDA, rat;* (33) 2. **No effect;** Rimonabant, WIN55, 212-2; *Acute NMDA, mouse;* (34)	No studies	1. **Prevented therapeutic effect of CBD;** Capsazapine; *Acute NMDA, mice;* (30)	No studies
Cognitive	1. **Prevention;** *Acute NMDA, rat;* (35) 2. **Prevention;** *SC/C NMDA, rat;* (36) 3. **Prevention;** *SC/C NMDA, mice;* (37) 4. **Reversal;** *SC/C NMDA, mice;* (38) 5. **Reversal;** *MIA, rat* (39, 40) 6. **Reversal;** *MAM, rat* (41) 7. **No effect;** *Acute NMDA, rat* (42) 8. **Negative effect;** *Unmanipulated rat;* (35)	1. **Prevention;** AM251; *SC/C NMDA, rat;* (43) 2. **Prevention;** AM251; *Acute NMDA, mice* (44) 3. **Reversal;** AM251; *SC/C NMDA, rat;* (45) 4. **No effect;** Oleamide; *Acute NMDA, mice;* (44) 5. **No effect;** AM251 *MAM, rat* (41) 6. **No effect on therapeutic effect of CBD;** AM251, AM630; *SC/C NMDA, mice;* (38)	1. **Prevention;** URB597; *Acute NMDA, mice;* (46) 2. **No effect;** URB597; *SC/C NMDA, rat;* (45) 3. **Negative effect;** JZL184; *Acute NMDA, mice;* (46)	No Studies	1. **Prevented negative effect of CBD;** WAY100635 *Untreated rat* (35) 2. **Prevented therapeutic effect of CBD;** WAY100635 *SC/C NMDA mice;* (38)
Social	1. **Prevention (intermediate dose);** *Acute NMDA, rat;* (32) 2. **Prevention;** SC/C NMDA, mice; (37) 3. **Reversal;** *SC/C NMDA, mice;* (38) 4. **Reversal;** *MIA, rat;* (39, 40) 5. **Reversal;** *MAM, rat;* (41) 6. **No effect (high dose);** *Acute NMDA, rat;* (32) 7. **No effect;** *Acute NMDA, rat* (47) 8. **Negative effect (low dose);** *Acute NMDA, rat;* (32) 9. **Negative effect;** *Untreated rat;* (47) 10. **Prevented therapeutic effect of antipsychotic;** *Acute NMDA, rat;* (47)	1. **Reversal;** AM251; *SC/C NMDA* (48) 2. **Reversal;** AM251; *MAM, rat;* (41) 3. **No effect;** AM251; *SC/C NMDA;* (45) 4. **Negative effect;** CP55,940; *Unmanipulated rat* (48). 5. **Prevented therapeutic effect of URB597;** AM251; *SC/C NMDA, rat;* (49)	1. **Reversal;** URB597; *SC/C NMDA, rat;* (45, 49) 2. **Negative effect;** URB597; *Unmanipulated rat;* (49, 50)	1. **Prevented negative effect of URB597;** Capsazapine; *Unmanipulated rat;* (49)	1. **Prevented therapeutic effect of CBD;** WAY100635 *SC/C NMDA; mice;* (38)

Bold typeface summarizes effect of experimental pharmacological treatment on the outcome measure. "Prevention" refers to preventing the induction of deficits by the experimental manipulation; "Reversal" refers to reversing deficits previously induced by the experimental manipulation; "Negative effect" refers to inducing or worsening deficits. In column 2, the experimental pharmacological treatment is CBD; in columns 3–6, the specific pharmacological treatment is specified for each entry. The experimental manipulation and animal species are in italic typeface: Acute NMDA antagonism (Acute NMDA), subchronic/chronic NMDA antagonism (SC/C NMDA), maternal immune activation (MIA), methylaxymethanol acetate treatment (MAM), or no experimental manipulation.

augmented cognitive effects of acute MK-801 challenge in this task (46). In male hooded Lister rats, MK-801 administered 30 min prior to testing induced working memory deficits in a delayed matching to position task; a crude CBD extract administered concomitantly with MK-801 failed to significantly reduce this effect (42). In male Sprague Dawley rats, MK-801 injected directly into the prefrontal cortex (PFC) induced deficits in attentional set-shifting, which were prevented by co-injection of CBD. CBD administered alone had an effect similar to MK-801, and this effect was prevented by concurrent infusion of a 5-HT1A/7 antagonist (35). Notably, the cognitive tasks used in these studies can be related to specific cognitive domains that are altered in schizophrenia (working memory, declarative memory and attentional set shifting).

Social Deficits

Acute MK-801 challenge (0.6 mg/kg) to male Sprague Dawley rats reduced the number of social encounters in the SI test (32), and a low dose of either CBD (3 mg/kg) or clozapine (1 mg/kg) prevented this effect, while higher doses (30 and 10 mg/kg, respectively) had no effect, nor did CBD given alone. In a separate study, CBD (3 mg/kg) prevented the effects of MK-801 (0.3 mg/kg) on social encounters, while a lower dose of CBD (1 mg/kg) potentiated the effects of MK-801 (53). In male Wistar rats, MK-801 (0.03–0.15 mg/kg) had no effect on social motivation, but social memory was impaired. This impairment was reduced by aripiprazole (an antipsychotic; 2 mg/kg), while risperodone and olanzapine had no effect. CBD did not prevent MK-801-induced deficits in social memory, and at high doses (12 and 30 mg/kg) impaired it. When CBD was given with aripiprazole, the effect of aripiprazole was lost (47). Methodological differences between these two groups—both with respect to experimental manipulation and outcome measures—make comparisons somewhat difficult, but it appears that MK-801 might have distinct dose-dependent effects on social behavior and social memory, and CBD, in turn, has distinct effects according to dose, outcome measure, and pharmacological context.

Subchronic/Chronic Challenge With NMDA Receptor Antagonist

Subchronic or chronic administration of ketamine, PCP, or MK801 has neurobehavioral effects that persist after drug washout. Many of these effects are similar to pathological characteristics of schizophrenia, including a reduction in hippocampal parvalbumin positive GABAergic interneurons, which is also observed in the prefrontal cortex in schizophrenia (54–58). Thus, repeated administration of NMDA receptor antagonists may replicate in rodents important neurobiological alterations of schizophrenia.

PPI

There is one published study on the effects of CBD on PPI deficits induced by chronic MK-801 treatment. MK-801 (0.1, 0.5, or 1.0 mg/kg) was administered across 14, 21, or 28 days, to 6 week old male C57/BL/6J mice. CBD (15, 30, 60 mg/kg) or clozapine (1 mg/kg) was co-applied beginning on day 6 of treatment. MK801 (1 mg/kg) for 28 days impaired PPI (measured 1 day after final MK-801 injection) and coadministration of either 60 mg/kg CBD or 1 mg/kg clozapine prevented this effect (31).

Cognitive Deficits

When MK-801 (0.5 mg/kg) was administered twice per day across 14 days to adult male C57BL/6J mice, deficits were observed in the NOR test 1 week after the final dose of MK801. These deficits were reversed by clozapine (1 mg/kg/day) or CBD (15 and 30 mg/kg/day, but no effect of 60 mg/kg/day), which were administered for 7 days following MK-801 treatment. The effects of CBD were prevented by the co-administration of WAY100635 (5-HT1A/7 antagonist), but not by co-administration of AM251 or AM630 (CB2 antagonist) (38). Gomes et al. (37), using the same chronic MK-801 treatment protocol applied to male C57/BL/6J mice described in the previous paragraph, reported that chronic MK-801-induced deficits in the NOR test were prevented by either co-administration of clozapine or 60 mg/kg CBD. In a separate study, adolescent male Lister-Hooded rats were treated for 5 successive days with PCP, followed by intermittent administrations across the next 3 weeks. Deficits in novel object discrimination were observed 3 days after the final PCP administration; these deficits were prevented by clozapine or AM251 (5 and 0.5 mg/kg, respectively, co-administered with PCP beginning on treatment day 10). PCP treatment increased 2-AG, but not anandamide levels in the prefrontal cortex, while AM251 increased anandamide levels when administered alone or when co-administered with PCP (43). Similarly, chronic ketamine administration (30 mg/kg/day for 10 days) to adult male Sprague Dawley rats caused deficits in the NOR test observed 7 or 14 days after the final dose of ketamine, while acute CBD administration (7.5 mg/kg just prior to the first NOR test) or subchronic CBD administration (7.5 mg/kg/day for 7 successive days) reversed this effect (36).

A single study (45) using this model and applying a cognitive test paradigm recommended by CNTRICS reported that PCP treatment (5 mg/kg, 2 times per day, for 7 days) in adult male Wistar rats caused a working memory deficit in a delayed alternation task 5 days after the final PCP dose. This deficit was reversed by AM251 (1 mg/kg), but not URB597 (an inhibitor of FAAH; 0.3 mg/kg), administered just before the task. Interestingly, both AM251 and URB597 caused working memory deficits when administered to rats that had not received PCP treatment. Taken together, studies of cognition in these models showed that deficits in object recognition and working memory were reversed and/or prevented by clozapine, AM251, or CBD. Therapeutic effects of CBD may be mediated by the 5-HT1A receptor. Notably, anandamide levels, or the relationship between anandamide and 2-AG levels, appeared to be associated with positive treatment response.

Social Deficits

In the studies of Rodrigues da Silva et al. (38) and Gomes et al. (37) described above, chronic MK-801 treatment resulted in reduced social interaction in male C57BL/6J mice that, along with cognitive deficits, were mitigated by either clozapine or CBD. The effect of CBD was prevented by WAY100635 administration

(38). Likewise, in the Seillier et al. (45) study described above, subchronic PCP treatment reduced social interaction between two freely interacting individuals that had received the same experimental treatment, in addition to inducing object memory deficits. However, unlike the cognitive deficits, social deficits were reversed by URB597, but not by AM251. In a later study by Seillier et al. (49) (same protocol as in 2010), it was reported that URB597 again reversed the social deficits induced by PCP, but induced social deficits in saline treated rats, a result that was replicated in a separate study (50). In PCP treated rats, the positive effect of URB597 was prevented by AM251, while the negative effect of URB597 in saline treated rats was prevented by capsazepine (a TRPV1 receptor antagonist). These results suggest that the positive effects of increasing anandamide levels within a pathological system may be mediated by the CB1 receptor, while negative effects of this same manipulation within a healthy system may be mediated by the by TRPV1 receptor. These investigators hypothesize that deficient CB1 receptor stimulation as a consequence of chronic PCP treatment is responsible for social withdrawal. Seillier and Guiffrida (48) next applied a modified versión of the social interaction test in which the focal animal interacted with untreated stimulus animals confined to wire mesh cages within the testing arena. This protocol allowed for distinguishing between social motivation and social recognition memory. PCP treated rats did not show differences from saline with respect to social motivation, but did show deficits in the capacity to distinguish between familiar and unfamiliar conspecifics. This effect of PCP was reversed by AM251, while in saline-treated rats, a CB1 agonist (CP55,940) induced social recognition deficits.

Neurodevelopmental Models

Maternal infection during pregnancy by certain viral pathogens is known to be a significant risk factor for schizophrenia in the offspring. Thus, risk for schizophrenia is increased by 2–7-fold from influenza infection during the first half of pregnancy (59). Several animal models have been developed in order to reproduce this risk factor; in general, these models involve gestational exposure to specific antigens that induce an immune response mimicking an infection (60). One such model that has been applied in the present context involves exposing the pregnant female rodent to polyinosinic:polycytidylic acid (Poly I:C), which is a compound structurally similar to viral RNA that stimulates an immune response similar to a viral infection. Notably, the results of these studies coincide well with those obtained using models based on NMDA receptor antagonism.

Osborne et al. (39, 40) applied a single dose of Poly I:C to pregnant Sprague Dawley rats on gestational day 15. Beginning on postnatal day 56, the adult male (39) and female (40) offspring were treated daily with CBD (10 mg/kg) or vehicle, and across days 72–80 were subjected to a NOR test, a delayed alternation test for working memory, and a social interaction test. Maternal immune activation resulted in deficits in object recognition and working memory, and decreased social interaction. All of these effects of maternal immune activation were reversed by CBD treatment.

A second neurodevelopmental model involves treating the pregnant dam with methylaxymethanol acetate (MAM), a DNA methylating agent, on gestational day 17. This treatment results in a number of neurobiological alterations in the adult offspring similar to those seen in schizophrenia (61). Stark et al. (41) found that CBD (30 mg/kg, but not 10 mg/kg) administered across postnatal days 19–39 reversed MAM-induced deficits in novel object discrimination in the NOR test as well as social interaction deficits. Interestingly, while neither AM251 nor haloperidol administered across the same postnatal period reversed NOR deficits, AM251 reversed social interaction deficits, and this effect was accompanied by a decrease in 2-AG levels in the prefrontal cortex.

FROM THE CLINIC TO THE LAB AND BACK AGAIN

Clinical trials suggest that CBD may be a useful pharmacotherapy for schizophrenia, but more studies are needed. Some clinical variables that are important to consider in future studies are disease chronicity (first episode vs. chronic disease), details of drug administration (alone or in combination with antipsychotics), and therapeutic objective [reducing existing symptoms or preventing disease progression; (62, 63)]. Likewise, in pre-clinical studies, these variables should be systematically considered within the experimental design. For example, acute NMDA receptor antagonist treatment can model the acute psychotic episode, while recent onset or chronic disease are best represented by persistent effects of neurodevelopmental challenges or subchronic or chronic administration of NMDA antagonists. CBD should be tested alone and in combination with distinct antipsychotics. [Indeed, at least one study indicates that CBD may reduce the effectiveness of antipsychotic treatment in an animal model (47)]. Possible preventative effects of CBD can be investigated by administering CBD before or along with the experimental challenge. More attention must be given to the comparability of the independent variables defined in animal model studies to controllable (and uncontrollable) clinical variables such as those mentioned above.

Likewise, outcome measures (dependent variables) should be standardized between pre-clinical and clinical studies. The present mini review discussed some outcome measures that are reasonably comparable between human and animal studies, and that have been applied in the laboratory (PPI and specific cognitive tests). The NOR, SI, and SR tests are highly practical for large scale use, but homologous tests for the clinic have not been developed. With respect to cognitive deficits, rodent tests that have clear human homologs, such as those identified by the CNTRICS initiative, should be more frequently applied. With respect to social deficits, new behavioral tests should be designed that more clearly capture specific domains of social dysfunction, perhaps including objective observations of human subjects in controlled social situations.

The body of studies presented here illustrates the general lack of comparability between pre-clinical and clinical studies. Of the clinical studies, only one (8) is reasonably comparable to just 4

(of 19) pre-clinical studies with respect to independent variables: CBD administered as a single drug to subjects with existing acute schizophrenia is comparable to CBD administration to rodent models that received subchronic/chronic NMDA receptor antagonist followed by drug washout (36, 38) or to neurodevelopmental models (39–41). Nevertheless, the outcome measures in these pre-clinical studies are not easily comparable to those of the Leweke et al. (8) study, except for deficits in social interaction, which are contemplated in the PANSS negative symptom subscale.

What are some other factors that may limit the translatability of pre-clinical results to the clinic? In the specific case of CBD and endocannabinoid modulators, responsiveness may be highly sensitive to dose. In the relatively few animal model studies where several different doses of CBD were tested, there was some indication that dose responsiveness to CBD might take the form of an inverted U, where lower and higher doses are ineffective or have negative effects. Given this information, it seems important that body mass be taken into consideration when analyzing clinical results, perhaps by including this factor as a covariable in the statistical analysis of the results. Secondly, clinical populations are likely heterogeneous with respect to underlying pathophysiologies, perhaps some being responsive to CBD or endocannabinoid system modulators, and others unresponsive. With this in mind, it might be useful to examine in an exploratory manner risk factor profiles of patient responders vs. non-responders, in order to define the dependent variables that are most appropriate for modeling responsiveness (and non-responsiveness) to CBD or endocannabinoid system modulators.

It is striking that the vast majority pre-clinical studies reviewed here were carried out using exclusively male animals. Human clinical populations obviously comprise both sexes, not to mention being more diverse than laboratory animals in terms of underlying pathology, age, diet, body weight, among many other characteristics. By contrast, laboratory rodent strains have been selectively bred for hundreds of generations and raised under highly controlled conditions, no doubt reducing genetic and epigenetic variability of the population. All of these factors could introduce variability into the clinical response that is not represented in pre-clinical studies, thus limiting the predictive power of the latter.

Since the results of clinical studies on a homogenous population would be almost meaningless in clinical practice, one possible way to address this limitation may be to systematically carry out pre-clinical experiments in mixed-sex animal populations that have more inherent genetic diversity, perhaps including rodent or non-rodent species that have not had an extended history of laboratory rearing, such as the prairie vole or deer mouse. Since important interspecies differences may exist with respect to pharmacological responsiveness [for example, rodent FAAH is inhibited by CBD, while the human counterpart is not (9)], it is advisable to investigate such details in a number of different species. In addition, a broader range of models should be employed: models based on different risk factors (distinct experimental manipulations) should be simultaneously tested, perhaps within the same experiment or through a coordinated multicenter effort, with the goal of approaching the diversity of the clinical population and potentially improving the predictive power of pre-clinical studies. Clearly, this strategy would require larger cohort sizes and strict multicenter coordination with regards to experimental design and outcome measures, the latter of which should be uniformly applied and readily comparable to outcome measures in clinical studies.

Animal models present an opportunity to examine in detail factors that might influence the therapeutic response to CBD in the clinic, as well as the physiological and neurobiological underpinnings of this response. In the case of cannabinoids or pharmacological manipulation of the endocannabinoid system as possible means to treat schizophrenia, studies on animal models indicate that CBD may be a useful pharmacotherapy, and suggest that pharmacotherapies that modulate anandamide and 2-AG levels should be developed and explored. Finally, studies on animal models point to interactions between cannabinoid receptors, the 5-HT1A/7 receptor, and the TRPV1 receptor that should be explored as possible targets for pharmacotherapy.

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A Research Domain Criteria Approach to Gambling Disorder and Behavioral Addictions: Decision-Making, Response Inhibition, and the Role of Cannabidiol

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Gambling Disorder (GD) has been recently re-classified in the DSM-5 under the "substance-related and addictive disorders," in light of its genetic, endophenotypic, and phenotypic resemblances to substance dependence. Diminished control is a core defining concept of psychoactive substance dependence or addiction and has given rise to the concept of "behavioral" addictions, which are syndromes analogous to substance addiction, but with a behavioral focus other than ingestion of a psychoactive substance. The main symptom clusters are represented by loss of control, craving/withdrawal, and neglect of other areas of life, whereas in a Research Domain Criteria (RDoC) perspective, GD patients exhibit deficits in the domain of "Positive valence systems," particularly in the "Approach motivation" and "Reward learning" constructs, as well as in the "Cognitive systems," primarily in the "Cognitive control" construct. In the Addictions Neuroclinical Assessment (ANA), three relevant domains for addictions emerge: "Incentive salience," "Negative Emotionality," and "Executive Function." The endocannabinoid system (ECS) may largely modulate these circuits, presenting a promising pharmaceutical avenue for treating addictions. Up to now, research on cannabidiol has shown some efficacy in Attention Deficit/Hyperactivity Disorder (ADHD), whereas in behavioral addictions its role has not been fully elucidated, as well as its precise action on RDoC domains. Herein, we review available evidence on RDoC domains affected in GD and behavioral addictions and summarize insights on the use of cannabidiol in those disorders and its potential mechanisms of action on reward, decisional, and sensorimotor processes.

Keywords: gambling disorder, behavioral addictions, endocannabinoids, Research Domain Criteria, cannabidiol

INTRODUCTION

Behavioral addictions refer to syndromes analogous to substance addiction, but with a behavioral focus other than ingestion of a psychoactive substance (1) and Gambling Disorder (GD) is often recognized as the prototypical behavioral addiction (2). The essential feature of behavioral addictions is the failure to resist an impulse, drive, or temptation to perform an act that is harmful to the person or to others. Each behavioral addiction is characterized by a recurrent pattern of behavior that has this essential feature within a specific domain. The repetitive engagement in these behaviors ultimately interferes with functioning in other domains. In this respect, the behavioral addictions resemble substance use disorders (1).

The diagnostic criteria for gambling disorder overlap largely with those for the substance use disorders: the main symptom clusters are represented by loss of control, craving/withdrawal, and neglect of other areas of life (3): there are commonalities between substance use disorders (SUDs), including the use of stimulants, alcohol, nicotine—and behavioral addictions including gambling, internet use, shopping, and eating, in terms of elements of automatized, dysregulated cognitions, and behaviors (4).

The inclusion of Gambling Disorder (GD) in the addictive disorder chapter of DSM-5 is motivated by the recognition of its genetic, endophenotypic, and phenotypic resemblances to substance dependence: both disorders show similar comorbidity patterns (5), genetic vulnerabilities, and responses to specific pharmacologic treatments (6).

The hallmark components of the disorder have been proposed to be (a) continued engagement in a behavior despite adverse consequences, (b) diminished self-control over engagement in the behavior, (c) compulsive engagement in the behavior, and (d) an appetitive urge or craving state prior to engaging in the behavior (7, 8).

Recently, a framework for an Addictions Neuroclinical Assessment (ANA) has been proposed (9). Three main neurofunctional domains, executive function, incentive salience, and negative emotionality, should be assessed in patients with addictions, including behavioral addictions ("process" addictions as defined by the American Society of Addiction Medicine, e.g., gambling) and in individuals at risk, for purposes of better understanding the heterogeneity of AD and eventually to improve the nosology.

The endocannabinoid system (ECS) has been shown to influence the acquisition and maintenance of drug-seeking behaviors, through its role in reward and brain plasticity. Cannabinoid receptors have been studied in addiction-related processes, with special attention paid to cannabinoid type 1 (CB1) receptors (CB1R). Other ionotropic cannabinoid receptors are also linked to neurophysiological functions in the ECS, such as transient receptor potential receptors, including transient receptor vanilloid potential 1 (TRVP1), which binds the endogenous cannabinoid anandamide (AEA) (10). Up to now, available evidence on the role of the ECS in GD and other behavioral addictions is still scarce and thus require a broadening of studies and a review of current results, in order to optimize treatment for those conditions and to consider the employ of cannabidiol and related compounds. In this review, we will briefly summarize the conceptualization of GD and behavioral addictions in a Research Domain Criteria (RDoC) framework, also considering the relevant neurocircuitry as candidate target for cannabidiol treatment and available evidence on the role of ECS and its dysregulations in those conditions.

GAMBLING DISORDER, BEHAVIORAL ADDICTIONS, AND THE RESEARCH DOMAIN CRITERIA

Gambling Disorder is characterized by a persistent, recurrent pattern of gambling that is associated with substantial distress or impairment. It is currently classified within the addictive disorder chapter of DSM-5 and it is characterized by a maladaptive pattern of gambling behavior that persists despite negative consequences in major areas of life functioning. GD is highly comorbid with other psychiatric disorders. The strongest evidence relates GD to substance use disorders: pathological gamblers have an increased risk of having a diagnosis of alcohol misuse in lifetime and an increased risk of having a substance use disorder (11). The National Institute of Mental Health (NIMH) has recently launched the Research Domain Criteria (RDoC) project to overcome the limitations of current classification systems and to develop a framework for research on mental disorders that includes multiple dimensions (12): behavior, thought patterns, neurobiological measures, and genetics, with a strong focus on neurocircuitries. The RDoC aims at facilitating the incorporation of behavioral neuroscience in the study of psychopathology and at identifying reliable and valid psychological and biological mechanisms and their disruptions, with an eventual goal of understanding how abnormalities in these mechanisms drive psychiatric symptoms (13). RDoC's strong focus on neural circuits is evident from the assumption that mental illnesses are conceptualized as brain disorders of brain circuits. Moreover, the RDoC assumes that dysfunctions in neural circuits can/will be identified by tools of neuroscience (12). Importantly, in the RDoC approach, the behavioral and genetic phenotypes are bridged and integrated through specific brain circuitries, which embody the level of systems biology (14–17). Recently, the RDoC matrix has been extended to include a sixth domain referred as "Sensorimotor Systems" which "*are primarily responsible for the control and execution of motor behaviors, and their refinement during learning and development*" (18). The belonging constructs seem to be related mainly to stereotypic behaviors and/or tics.

Neurocircuitries are phenotypic targets of great potential for endophenotypic/biomarker discovery in current neuroimaging clinical research (19). In a RDoC perspective, patients with behavioral addictions—and GD—exhibit impairments in the domain of "Positive valence systems," particularly in the "Approach motivation" and "Reward learning" constructs, as well as in the "Cognitive systems," more specifically in the "Cognitive control" construct. Patients with Attention Deficit/Hyperactivity Disorder (ADHD) seem to display, as well, impairments in the domains of

A
Domain: Positive Valence Systems

Construct	Subconstruct
Approach motivation	**Reward valuation**
	Effort valuation / willingness to work
	Expectancy / reward prediction error
	Action selection / preference-based decision making
Initial responsiveness to reward attainment	
Sustained / longer-term responsiveness to reward attainment	
Reward learning	
Habit	

B
Domain: Cognitive systems

Construct	Subconstruct
Attention	
Perception	
Declarative memory	
Language	
Cognitive control	Goal selection
	Response inhibition
	Performance monitoring
Working memory	

FIGURE 1 | RDoC domains involved in GD. **(A)** Positive valence systems, **(B)** cognitive systems (adapted from: NIMH RDoC Matrix https://www.nimh.nih.gov/research-priorities/rdoc/constructs/rdoc-matrix.shtml).

"Positive Valence Systems" (Reward anticipation, receipt, and delay) and "Cognitive systems" (Working memory) (20) (**Figure 1**).

Positive valence systems are primarily responsible for responses to motivational situations such as reward seeking, consummatory behavior, and reward/habit learning (18). The construct of Approach motivation involves "*mechanisms/processes that regulate the direction and maintenance of approach behavior influenced by pre-existing tendencies, learning, memory, stimulus characteristics, and deprivation states*" (ibidem). Particularly relevant to GD is the subconstruct Reward valuation, which consists of "*processes by which the probability and benefits of a prospective outcome are computed and calibrated by reference to external information, social context (e.g., group input, counterfactual comparisons), and/or prior experience. This calibration is influenced by pre-existing biases, learning, memory, stimulus characteristics, and deprivation states. Reward valuation may involve the assignment of incentive salience to stimuli*" (ibidem).

Cognitive systems are responsible for various cognitive processes. Specifically, cognitive control "*modulates the operation of other cognitive and emotional systems, in the service of goal-directed behavior, when prepotent modes of responding are not adequate to meet the demands of the current context. Additionally, control processes are engaged in the case of novel contexts, where appropriate responses need to be selected from among competing alternatives*" (21).

A complementary initiative to the RDoC is the Addictions Neuroclinical Assessment (ANA) (9), that incorporates key functional domains derived from the neurocircuitry of addiction. In this one, three domains (executive function, incentive salience, and negative emotionality) tied to different phases in the cycle of addiction, form the core functional elements of addictive disorders. The common point between RDoC and ANA is the consideration of neuroscience domains and the identification of meaningful subtypes of disorders.

GAMBLING DISORDER DOMAINS: BEHAVIORAL TASKS AND NEUROCIRCUITRY

Positive Valence Systems

Approach Motivation: Preference-Based Decision-Making

In a RDoC perspective, these processes involve an evaluation of costs/benefits and occur in the context of multiple potential choices being available for decision-making (18).

Changes in reward based decision-making and increases in impulsivity are hallmark features of addiction (22) that has been scarcely studied satisfactorily in GD. Risky decision-making is a core feature of GD: gamblers have a high tolerance toward risk (23, 24) and a bias to select short-term over long-term rewards is integral to the syndrome (25). This bias has been operationalized with the employ of a behavioral measure called delay discounting task [DDT; (26)], in which participants choose between pairs of options that yield small, immediate vs. large, delayed rewards. Subjects with substance abuse and behavioral addictions show a tendency to choose small and immediate rewards rather than large and delayed rewards. The Iowa Gambling Task (IGT) (27) has also been employed as a measure of decision-making, since it is considered as the most widely used and ecologically valid measure of decision making in this clinical population. In the IGT, players are given four decks of cards and an endowment of fake money (e.g., $2,000) and are instructed to select cards one at a time and try to lose the least amount of money and win the most. GD subjects have shown to perform worse on the IGT and to make more high-risk choices compared to controls, precisely after experiencing wins and losses (28). During high-risk gambling decisions, fMRI has shown that, during the IGT task, GD subjects exhibit relatively increased frontal lobe and basal ganglia activation, particularly involving the orbitofrontal cortex (OFC), caudate and amygdala. Increased activation of regions encompassing the extended reward pathway in GD subjects (GDs) during high risk choices suggests that the persistence of GD may be due to the increased salience of immediate and greater potential monetary rewards relative to lower monetary rewards or potential future losses (ibidem). There is also considerable evidence that GDs discount delayed rewards steeper than healthy controls (29). Neuroimaging research has shown that GD is associated with a shift in the

interplay between a prefrontal-parietal control network and a brain network involved in immediate reward consumption (30), and a generally hypoactive reward system (31).

A differential activation of distinguishable neural systems between immediate and delayed choices has been highlighted, with the former driven by the limbic system (including the ventral striatum, medial orbitofrontal cortex (MOFC), medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC), and left posterior hippocampus) and the latter by the lateral prefrontal cortex and associated structures [including the right and left intraparietal cortex (RPar, LPar), right dorsolateral prefrontal cortex (DLPFC), right ventrolateral prefrontal cortex (VLPFC), and right lateral orbitofrontal cortex (LOFC)] (32).

More specifically, there is evidence that the right hemisphere plays an important role in inhibiting impulsive behavior and that the right DLPFC holds a certain role in the process of general decision-making (33). Although the pathophysiology of GD is not well-understood, studies have shown altered brain activity in prefrontal regions (primarily the DLPFC) of GD patients in response to gambling stimuli. Recently, a hypersensitivity to extreme gain–loss ratios of dorsal cortico-striatal network involved in action–outcome contingencies has been shown in gamblers (34).

Reward Learning

The similarity between GD and substance abuse has been repeatedly hypothesized on the basis of large overlaps between addictive manifestations of both disorders. Recently, an interesting contribution to a broader understanding of the neurocognitive features of GD, hypothesized a loss of willpower to resist gambling, deriving from a pathological usurpation of mechanisms of learning that under normal circumstances serve to shape survival behaviors related to the pursuit of rewards and the cues that predict them (35). This mechanism has been shown to be related with reward-based cognitive inflexibility, presumably resulting from an aberrant reward-based learning and observed as some kind of continuous gambling even in the face of increasing losses (36).

On a neurobiological perspective, reward-based cognitive inflexibility, has been associated with the orbitofrontal cortex (OFC) (37), the ventral prefrontal cortex (vPFC) (38), the ventrolateral prefrontal cortex (vl-PFC) (39) and is facilitated by dopaminergic activity in the ventral regions of the striatum (37, 38).

Cognitive Control

Response Inhibition

Response inhibition refers to the ability to suppress behaviors that are inappropriate, unsafe, or no longer required (40). Recent findings suggest that the ability to suppress automatic responses could be critical to gambling addictive behavior (35). Whereas the increased sensitization toward gambling-related cues appears to be related to a hyperactivity of impulsive processes that may explain gamblers' motivation to seek out relevant reward (35), the unsuccessful efforts to reduce or stop gambling despite negative outcomes (19, 41–43) are thought to depend on a dysregulation of the so-called "reflective system," and specifically, a faulty inhibitory control, responsible for inadequate efforts to control (or cut back or stop) gambling (ibidem).

Inhibitory control has been usually assessed with behavioral measures such as the Stop Signal Task (SST) (44), in which subjects perform a choice reaction task, and, on a random selection of the trials, an auditory stop signal instructs subjects to withhold their response, or Go/No-Go tasks, which require people to make manual responses to rapidly presented visual or auditory cues (i.e., "Go" stimuli), but to withhold responses in the presence of a different cue ("No-Go" stimuli) (45).

Deficits in behavioral and cognitive control constitute a symptom dimension associated with diminished response inhibition in experimental tasks. Impaired response inhibition performance (i.e., prolonged latency of motor response inhibition) has been previously highlighted in pathological gambling by using the stop-signal task and the go/no-go paradigm [for a review, see (35)] and recent contributions highlight the correlation between deficits in response inhibition and gambling severity (46, 47).

Recent neuroimaging research suggests that response inhibition may depend on a fronto-basal-ganglia circuit, including the inferior frontal gyrus (IFG), the pre-supplementary motor area (pre-SMA) and the subthalamic nucleus (STN) and striatum (48). Both right IFG and pre-SMA activation appear to be associated with successful stop trials. However, whereas right IFG contributes to response inhibition and not to monitoring performance or adjusting behavior, the pre-SMA seem to be involved in monitoring or resolving the conflict between the opposing task demands in the stop-signal paradigm. Also, fMRI studies showed inhibition-related activation in basal ganglia, including the STN and striatum and lesions to the basal ganglia impaired stop performance for both humans and rodents (ibidem).

The concept of "loss of control" (LOC) reflects a psychopathology construct that is uniquely associated with distress and impairment and that, in eating disorders, is defined as a subjective experience of loss of control irrespective of the actual amount of food consumed (49). LOC has been extensively investigated in other consummatory behaviors, such as eating behaviors, where LOC frequently occurs in response to negative emotions in youth and then in adults, is associated with emotional disregulation (ibidem).

The construct of LOC is also closely related to the concept of "perceived control," since even with the absence of objective control, having the perception of control is sufficient to increase arousal and mobilize action; whereas perceiving the lack or loss of control leads to helplessness despite the presence of objective control (50). On the other hand, a crucial role in the loss of control is the motor component, which reflects the construct of inhibitory control and is associated with decreased functionality of the prefrontal cortex, which involves an impaired ability to control behaviors (51–53). Disruption of the PFC in addiction underlies not only compulsive drug taking but also accounts for the disadvantageous behaviors that are associated with addiction and the erosion of free will (53). The role of inhibitory control in relation to the development and maintenance of loss of control over behavior is still to be fully elucidated, as well as the

role of automatic processes as potential mediating factors (54). Herein, we focused on the symptom cluster "loss of control" (i.e., unsuccessful efforts to control, cut back, or stop gambling), which appears to be mainly related to impaired reward-related decision-making and deficits in executive functions. What is crucial to understand in regard to behavioral addictions is which component of LOC is predominant and in which phase of addiction and, more important, if there is any specificity for the affective or motor dimension to certain behavioral addictions. This could help in dissociating the neurocircuitry for those disorders, focusing more on reward-related-basal ganglia loops or on the prefrontal-orbitofrontal networks.

THE ENDOCANNABINOID SYSTEM

The endocannabinoid system (ECS) is a widespread neuromodulatory system that plays important roles in central nervous system (CNS) development, synaptic plasticity, and the response to endogenous and environmental insults. The ECS is comprised of cannabinoid receptors, endogenous cannabinoids (endocannabinoids), and the enzymes responsible for the synthesis and degradation of the endocannabinoids. perturbations of the ECS are involved in several psychiatric disorders, including schizophrenia (55). The most relevant receptors are CB1R and CB2R: while CB1R are abundant in the central nervous system (CNS), particularly in cortex, basal ganglia, hippocampus, and cerebellum, CB2R are expressed at much lower levels in the CNS compared to CB1R, and are primarily present in microglia and vascular elements (ibidem). The compound Δ9-tetrahydrocannabinol (THC) is the main psychoactive compound of *Cannabis sativa* L., whereas cannabidiol is one of the most abundant phytocannabinoids isolated from *Cannabis sativa L.* (up to 40% of the extract). In contrast with THC, cannabidiol does not exhibit psychomimetic activities. Several studies show CBD to have anti-inflammatory, anticonvulsant, antioxidant, antiemetic, anxiolytic, and antipsychotic properties; thus, it may serve as potential drug for the treatment of neuro-inflammation, epilepsy, oxidative injury, vomiting and nausea, and anxiety and schizophrenia, respectively (56).

Endocannabinoid Signaling and Reward

Both exogenous AEA and 2-arachidonoyl glycerol (2-AG) increase extracellular dopamine levels in the nucleus accumbens in a CB1R-dependent manner and the ECS exerts a strong influence on the fine-tuning of midbrain dopamine cell activity. Through these and other interactions the ECS has a prominent influence on the hedonic effects of natural rewards such as food, sexual activity, and social interaction. This is mediated in part through a direct CB1R modulation of the mesolimbic dopamine response to natural reward and through the interactions between the ECS and other signaling systems (endogenous opioids, hypothalamic signaling molecules, etc.) (57). Although enhancement of endocannabinoids (EC) levels does not produce rewarding effects *per se*, EC signaling at cannabinoid receptors participates in the mediation and modulation of both natural and drug-induced reward. Brain EC content is modulated by most drugs of abuse and natural rewards and a robust CB1R influence on the motivation to consume distinct classes of abused drugs and the association of CNR1 gene polymorphisms with aberrant reward processing and addictive behaviors strongly implicates CB1Rs in the etiology of addiction (ibidem). Also several studies have suggested an association between acute or chronic use of exogenous cannabinoids (THC) and executive impairments, and a relevant modulation of the endocannabinoid system on prefrontal-dependent cognitive functioning and executive functioning has been highlighted (58).

THE ENDOCANNABINOID SYSTEM AND THE RDoC

Endocannabinoid functioning has been recently studied in a RDoC perspective (59): its role in Positive Valence Systems and Cognitive Systems has been highlighted. Specifically, *reward attainment* is one of the only RDoC constructs to explicitly detail endocannabinoids as candidate modulators of reward learning, valuation, and processing (ibidem). In regard to Cognitive systems and particularly, *declarative memory*, stimulation of cannabinoid receptors in hippocampal circuits diminishes glutamate release to below-threshold levels, inhibiting long-term potentiation necessary for encoding and abundance of evidence demonstrates transient, dose-dependent Δ9-tetrahydrocannabinol (THC)-induced memory impairments (with a tolerance effect in heavy users) and the contrasting absence of memory deficits following CBD administration. THC exposure in humans negatively impacts working memory *via* CB1R activation and inhibition of AEA reuptake. Correspondingly, rodent models with upregulated CB1R expression in the PFC, as well as CB1R knockout mice, demonstrate changes in cognitive flexibility. Low doses of CB1R antagonists improved task switching (a measure of cognitive flexibility) and inhibitory control *via* inhibition of PFC glutamatergic activity, whereas CB1R agonists increased impulsive behaviors. A neuroimaging study suggests that THC impacts activity in cerebral inhibition response circuits causing increased hyperactivity in the PFC and anterior cingulate cortices. Acute administration of THC reduces response inhibition (that is, increases behavioral impulsivity) and causes hyperactivity at dopaminergic synapses in the PFC (59) (**Figure 2**).

THC also induces impairments in decision-making, which are thought to be the result of cannabinoid CB1R activation (60). In rat model of IGT (rat gambling task—rGT), blockade of the CB1R produced a trend improvement in decision making in animals who preferred the advantageous task options, yet left choice unaffected in risk-prone rats. Neither CB1R agonist had strong effects on decision-making, but a high dose THC decreased premature responses (ibidem). These results show that acute modulation of CB1R has modest effects on choice and instead may play a substantive role in regulating impulsive responding. Animal models also shown that activation of the cannabinoid system in the nucleus accumbens (NAc) is capable to impair effort-based decision-making: rats trained in a T-maze

RDoC domain	RDoC construct	EC system signaling
Positive valence systems	Reward learning	AEA mobilization in the NAc and hippocampus via activation of OT receptors
	Motivation	Enhanced motivation is observed following CB1R stimulation and with increased AEA signaling in the amygdala, NAc and dorsal striatum and 2-AG in the NAc. Diminished motivation is associated with CB1R blockade.
Cognitive systems	Declarative memory	AEA and 2-AG are both robust modulators of early-stage acquisition, consolidation and extinction. Enhancement of 2-AG is correlated with disrupted encoding in spatial memory.
	Working memory	Low doses of CB1R antagonists improved task switching (a measure of cognitive flexibility) and inhibitory control via inhibition of PFC glutamatergic activity, whereas CB1R agonists increased impulsive behaviors. A neuroimaging study suggests that THC impacts activity in cerebral inhibition response circuits causing increased hyperactivity in the PFC and anterior cingulate cortices.

FIGURE 2 | EC systems signaling involved in specific RDoC domains and constructs.

cost-benefit decision making task were led to be less willing to invest the physical effort to gain large reward after administration of cannabinoid system agonist (61). The anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) are also involved in decision-making and murine models employing cost-benefit T-maze decision-making task showed that CB1R activation in the ACC impaired decision making such that rats were less willing to invest physical effort to gain high reward. Similarly, CB1R activation in the OFC induced impulsive pattern of choice such that rats preferred small immediate rewards to large delayed rewards (62).

CANNABIDIOL AND RESPONSE INHIBITION

Response inhibition, as mentioned before, refers to the ability to suppress behaviors that are inappropriate, unsafe, or no longer required (40). Whereas THC impairs performance on motor and response inhibition tasks, cannabidiol (CBD) does not impair motor or cognitive performance (63). The Go/No-Go task is a classical response inhibition paradigm that requires participants either to execute or inhibit a motor response and recent contributions have examined the differential effects of Δ-9-THC and CBD on regional brain activation during response inhibition tasks. In regard to the specific behavioral response, neither THC nor CBD had a significant effect on task performance, save for an effect on the frequency of left/right errors (ibidem). A previous study (64) investigated the acute effects of THC on four behavioral measures of impulsivity (including a Go/No-Go task) in recreational marijuana users. THC impaired performance on a Stop task but did not have a significant effect on Go/No-Go performance, suggesting that THC may increase certain forms of impulsive behavior more than others. However, it is suggested that THC attenuates the engagement of brain regions that mediate response inhibition and that CBD modulates function in regions not usually implicated in response inhibition.

Another study investigated the differential effects of Δ-9-THC and CBD on regional brain activation during a set of four tasks that engaged cognitive processes known to be affected by cannabis use: verbal memory, response inhibition, sensory processing, and emotional processing (65). Specifically, response inhibition was measured with the Go/No-Go task and opposite effects of THC and CBD were observed in the parahippocampal gyrus during response inhibition. Although the parahippocampal areas are not part of the response inhibition network, opposite effects of Δ-9-THC and CBD in the parahippocampal areas during the response inhibition task is consistent with the high density of CB1R in these regions (ibidem).

Animal models have been employed to study the role of the ECS in response inhibition: CB1R agonists and antagonists were tested in rats during a stop-signal paradigm (the main task employed to test response inhibition). Results showed that while response inhibition has been shown to be impaired in human volunteers after THC administration, neither disruption of endocannabinoid signaling nor administration of a CB1R

agonist had clear observable behavioral effects on stop-signal task performance (66). Differential effects on adolescent mice have been shown by pharmacological inhibition of the fatty acid amide hydrolase (FAAH), the major enzyme implicated in anandamide degradation. Murine models showed that it prevented cognitive disruptions induced by distracting cues in adolescent mice. In particular, these protective effects were indicated by increased accuracy and correct responses and decreased premature responses selectively in the distractor trials (67).

CANNABINOIDS IN NEUROPSYCHIATRIC DISORDERS CHARACTERIZED BY IMPULSIVITY AND RESPONSE INHIBITION IMPAIRMENTS

In the last decade, a number of studies investigated the use of CBD in neuropsychiatric disorders characterized by motor and cognitive impulsivity/compulsivity, such as Attention Deficit/Hyperactivity Disorder (ADHD) and Tourette syndrome. In regard to ADHD, it is known that two regions of the endocannabinoid system, the hippocampus and cerebellar vermis, have been identified as being uniquely influenced by an interaction between cannabis use and the altered brain circuitry of ADHD diagnosed individuals and in a recent study (68). ADHD participants had impaired response inhibition combined with less fronto-parietal/striatal activity, regardless of cannabis use history and cannabis use did not impact behavioral response inhibition. Also, cannabis use was associated with hippocampal and cerebellar activation, areas rich in cannabinoid receptors, in control group but not ADHD participants (ibidem). Also, a childhood diagnosis of ADHD, but not cannabis use in adulthood, was associated with executive dysfunction. Earlier initiation of cannabis use may be linked to poor cognitive outcomes and a significantly greater proportion of the ADHD group began using cannabis before age 16. Regular cannabis use starting after age 16 may not be sufficient to aggravate longstanding cognitive deficits characteristic of ADHD (69).

In Tourette syndrome, Δ9-THC efficaciously reverses peripheral but not central motor tics. Δ9-THC may reduce ambulatory movements and evoke premonitory urges in some pediatric patients. The small "therapeutic window" in juveniles suggests that CBD may not effectively treat motor tics in children and may even exacerbate tics in a population of patients with Tourette syndrome (70). However, a recent systematic review suggests that there is insufficient evidence to provide guidance on the use of cannabinoids for mental health conditions within a regulatory framework, since only a single, small RCT for ADHD compared pharmaceutical THC:CBD with placebo and no significant effect was seen on the primary outcome, ADHD symptoms (71). Also, two small studies demonstrated no significant benefit of pharmaceutical THC:CBD compared to placebo on Tic/Tourette symptoms (ibidem).

CANNABIDIOL AS A CANDIDATE TREATMENT FOR ADDICTIONS AND DISORDERS OF MOTIVATION

Cannabidiol (CBD) is one such drug that shows therapeutic potential in a broad range of neurological and psychiatric diseases. Emerging preclinical and clinical evidence also indicates that CBD regulates different aversive and appetitive memory processes (10, 72). In preclinical studies in humans and animals, CBD reduces drug-motivated behavior, attenuates withdrawal effects, and limits cravings. Consistent with results demonstrating antagonizing effects of CBD on THC-induced pharmacological actions, cannabis containing higher vs. lower levels of CBD decreases the incentive salience of cannabis-related stimuli in smokers, and a case study reported a reduction in cannabis withdrawal symptoms following CBD administration (73). In contrast to its effects on opioid-motivated behaviors, CBD has less apparent influence on psychostimulant reward and reinforcement (ibidem). The endocannabinoid system might be of relevance to impulsivity and decision-making. Administration of high doses of CB1R agonists increases impulsive behaviors, whereas the administration of low doses of CB1 antagonists improves set-shifting performance and reduces the number of impulsive responses (74). In a rat model of gambling disorder, the administration of a CB1/2 agonist improved choice performance in a suboptimal group of rats, as evaluated using the rat gambling task (rGT). Although it is premature to propose that the stimulation of CB1/2R may provide a treatment for gambling individuals prone to poor decision-making the study from Gueye and colleagues implicates the cannabinoid system in the processing of cost-benefit decision-making. It should be noted that, up to date cannabidiol (or cannabidiol/THC mixtures) have mainly been studied in substance use disorders: CBD and THC mixtures showed positive effects in reducing short-term withdrawal and craving in cannabis use disorders, while studies on schizophrenia and comorbid substance use are lacking (75). Currently, there are only clinical studies on substance use disorder, while the effects of cannabidiol in other types of addiction or disorders of motivation have not been studied in randomized clinical trials yet.

CONCLUSIONS

The inclusion of GD in the "substance related and addictive disorders" chapter of DSM-5 recognizes the disorder as a prototypical behavioral addiction, characterized by symptom clusters of loss of control, craving/withdrawal, and neglect of other areas of life.

The adoption of a RDoC approach facilitates the identification of the neurobiological factors underlying the disorder by breaking up a complex psychiatric disorder into its components and domains and identifying the corresponding constructs and subconstructs, thus rendering the process more tangible and experimentally addressable. Importantly, RDoC constructs relate to biological and behavioral measures and may also help in identifying endophenotypes for the disorder. Therefore,

recent research in GD is focusing on the identification of the neurobiological underpinnings of most employed behavioral tasks related to decision making and response inhibition (e.g., Iowa Gambling Task, Delayed Discounting Task, and Stop Signal Task), to identify the neural correlates of the disorder's symptomatologic clusters and domains.

These deficits are associated with the RDoC domains of Positive Valence Systems (and its constructs of Approach motivation and Reward learning) and Cognitive Control (mainly its construct Response inhibition), respectively. Consistent with the RDoC matrix, deficits in preference-based decision-making have been identified in GD with the utilization of the IGT, revealing an involvement of numerous brain areas such as the striatum, amygdala, and OFC. Evidence regarding aberrant reward learning mechanisms are less robust, nevertheless they were hypothesized to be related with reward-based cognitive inflexibility and associated with an involvement of the OFC and ventral striatum, as highlighted in the RDoC matrix. Lastly, deficits in Cognitive control and particularly in the subconstruct of response inhibition have been identified in the disorder, using the SST and the Go/No-Go task, revealing the involvement of a fronto-striatal circuit and of the pre-supplementary motor area (pre-SMA). Further research is needed to expand our knowledge regarding the constructs of the disorder and how they correlate with the clinical presentation of the disorder as well as with the abnormalities at a neurocircuits level of explanation. The endocannabinoid system has been shown to play a crucial role in the regulation of different aversive and appetitive memory processes related to addiction mechanisms. In a RDoC perspective, EC role has been highlighted in Positive Valence Systems (reward attainment) and Cognitive Systems (declarative memory and working memory) has been highlighted. A putative role of endocannabinoids in response inhibition mechanisms has also been hypothesized, deriving evidence from the use of CBD during the Go/No-Go task. Nevertheless, evidence is still scarce to clearly determine which disorders may benefit from CBD administration based on impaired RDoC domains and constructs. Some insights derive from studies conducted on neuropsychiatric disorders characterized by motor and cognitive impulsivity and deficits in executive functions and response inhibition (e.g., ADHD, Tourette syndrome). This might also lead to hypothesize an involvement of EC system in the new sensorimotor domain of RDoC. Animal and human neuroimaging studies have also shown differential effects of THC vs. CBD on specific tasks and regional brain metabolism and, especially, in specific sub-populations. This might reflect the case of other compounds and substances, such as caffeine, whose effects clearly depends on the age window of administration. What is crucial to consider in this context is the developmental trajectory of the disorder: studies in this field have already unraveled the this dimension for response flexibility—an executive function that resembles simple motor inhibition in that both depend on sustained attention and the inhibition of prepotent responses, that differs from motor inhibition in that only the former requires subjects to execute an alternative response when the appropriate cue appears—in bipolar disorder (76). The evidence of differences in cognitive control between children and adults has also been highlighted by fMRI studies showing that children are more susceptible to interference and in prefrontal function and improvements in cognitive less able to inhibit inappropriate responses than adults. Effective interference suppression in children was associated with prefrontal activation in the opposite hemisphere relative to adults. In contrast, effective response inhibition in children was associated with activation of posterior, but not prefrontal, regions activated by adults. Children failed to activate a region in right ventrolateral prefrontal cortex that was recruited for both types of cognitive control by adults. Thus, children exhibited immature prefrontal activation that varied according to the type of cognitive control required (77). These differences may account for the differential choice of a specific compound that may exert an effect on the trajectory of development of brain networks and neurotransmettitorial signaling only in certain age groups.

More recently in the field of behavioral addictions, other contributions have disentangled similar mechanisms of faulty inhibitory control and faulty decision-making with preference for immediate reward to long-term gains in subjects with Internet gaming disorder (IGD) (78). Specifically, IGD subjects performing the Go/No Go task in fMRI showed greater impulsivity and lower activity of the right supplementary motor area/presupplementary motor area and showed increased activation in orbito-frontal cortex in gain trials and decreased anterior cingulate cortex activation in loss trials implicating enhanced reward sensitivity and decreased loss sensitivity (ibidem). Furthermore, regular or chronic IGD resulted in reduced brain's dopamine indicated by lower dopamine transporter density and lower dopamine D2 receptor occupancy in the brains of videogame players. In summary, further research is needed to elucidate the potential mechanisms involved in the regulation of response inhibition and reward-related decision-making that may be partially or fully mediated by EC system in behavioral addictions and, more specifically, in GD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SP, AM, and NM have contributed to the ideation, drafting, and preparation of the paper. All authors contributed to the article and approved the submitted version.

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