

# At the Tip of an Iceberg: Prenatal Marijuana and Its Possible Relation to Neuropsychiatric Outcome in the Offspring

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## ABSTRACT

Endocannabinoids regulate brain development via modulating neural proliferation, migration, and the differentiation of lineage-committed cells. In the fetal nervous system, (endo)cannabinoid-sensing receptors and the enzymatic machinery of endocannabinoid metabolism exhibit a cellular distribution map different from that in the adult, implying distinct functions. Notably, cannabinoid receptors serve as molecular targets for the psychotropic plant-derived cannabis constituent  $\Delta^9$ -tetrahydrocannabinol, as well as synthetic derivatives (designer drugs). Over 180 million people use cannabis for recreational or medical purposes globally. Recreational cannabis is recognized as a niche drug for adolescents and young adults. This review combines data from human and experimental studies to show that long-term and heavy cannabis use during pregnancy can impair brain maturation and predispose the offspring to neurodevelopmental disorders. By discussing the mechanisms of cannabinoid receptor-mediated signaling events at critical stages of fetal brain development, we organize histopathologic, biochemical, molecular, and behavioral findings into a logical hypothesis predicting neuronal vulnerability to and attenuated adaptation toward environmental challenges (stress, drug exposure, medication) in children affected by in utero cannabinoid exposure. Conversely, we suggest that endocannabinoid signaling can be an appealing druggable target to dampen neuronal activity if pre-existing pathologies associate with circuit hyperexcitability. Yet, we warn that the lack of critical data from longitudinal follow-up studies precludes valid conclusions on possible delayed and adverse side effects. Overall, our conclusion weighs in on the ongoing public debate on cannabis legalization, particularly in medical contexts.

**Keywords:** Epilepsy, Marijuana, Psychosis, Schizophrenia, Skunk, Stratification

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The developing central nervous system (CNS) relies on a wide array of signaling mechanisms with their precisely orchestrated cross-talk shaping a combinatorial code for neuronal development. This temporally defined procedure is reflected in the production, differentiation, and migration of neurons and glial cells (1–3). In addition to genetic predisposition, harmful environmental agents can potentially impact brain development. These include alcohol and nicotine as unregulated substances and many types of illicit drugs. Marijuana (*Cannabis sativa*), its selectively cultivated subspecies (e.g., skunk), and its synthetic derivatives (designer drugs) are commonly consumed (4,5). According to the US Substance Abuse and Mental Health Services Administration, their use peaks between 15 and 30 years of age with a trend for continued consumption by people aged 30 to 40 years and over (6–8). Due to penal sanctions for cannabis possession and abuse in several European countries (9), potent synthetic cannabinoids, mimicking or amplifying psychoactive effects of  $\Delta^9$ -tetrahydrocannabinol (THC), are offered as legal alternatives (10). The prevalence of synthetic cannabinoid use by adolescents is significantly higher in the United States (7.4%) than in European countries (e.g., .2% and 1.4% in the United Kingdom

and Spain, respectively) without further increases in recent years (10). Alarming, cannabinoids are the substance chiefly abused by pregnant women: its prevalence exceeds 10% in the United States (7), while it remains largely unknown yet with predicted socioeconomic variability (1% to 16%) in Europe (9). This generation-driven pattern of cannabis use exposes the brain to THC during at least one critical developmental period: in utero development of fetus, childhood, or teenagehood (11).

A growing body of evidence demonstrates that agents that are generally considered moderately harmful to the mother when consumed at limited quantities (e.g., alcohol, nicotine, morphine, or cannabis) may pose severe threats—unrelated to miscarriage or placental deficits—to the fetus, providing the foundation of neurobehavioral teratology (12). CNS vulnerability is a leading fingerprint of harmful developmental drug effects and predominantly manifests as functional impairments in early childhood or adolescence, much less so by birth (13). These observations fuel the double-hit hypothesis that defines subthreshold stimuli as triggers of severe malfunction of sensitized yet nonsymptomatic neuronal circuits with often considerable delay in postnatal life (14,15). Nevertheless,

cannabis-induced early prenatal lethality could be underestimated. Rodent and chick experiments show that marijuana embryotoxicity manifests as neural plate aplasia at early intrauterine time points, which, when considering their human equivalents (gestational days 15–19), would likely be clinically misinterpreted as a lack of embryo implantation (16).

In addition to existing sociopolitical, economical, and ethical arguments, recent campaigns aimed to decriminalize cannabis were motivated by current patterns of use and comparisons to alcohol, tobacco, heroin, and methamphetamine citing small-to-moderate adverse public health impact for cannabis (17). This view is particularly prevalent since this “soft drug” (18,19) causes seemingly reversible effects on cognitive abilities after abstinence (20) and leads to psychotic outcomes without relapse in adults (21). Case-control studies in the United Kingdom, however, indicate that high-potency cannabis variants (e.g., skunk) triple the risk (at earlier onset) of psychosis (22). Conversely, cannabidiol, a nonpsychotropic cannabis constituent, is reported as being a potent antipsychotic agent and indicated for disease treatment (23,24) since it appears to reduce the psychotropic action of THC (25). Magnetic resonance imaging showed that neither volumetric nor shape-based measures of brain regions driving conscious behavior are altered by daily marijuana use (26). Moreover, and for human offspring, prenatal marijuana does not induce gross anatomical deformities or deficits in vital functions directly by or after birth (27–29). This lends support to the double-hit hypothesis of THC-induced neuronal sensitization (but see embryotoxicity above), which is favored by THC’s efficacious cross-placental transfer and excretion during lactation (30). As such, THC concentration of breast milk in humans may be up to eightfold higher than simultaneously measured maternal plasma concentrations (31). Therefore, it is plausible that continued maternal cannabis use during the first months postpartum could evoke neurological consequences in toddlers by 1 year of age (32) or later.

A more indirect way of cannabinoids to compromise pregnancy outcome and fetoplacental development is through their effect on maternal and placental hormone signaling. In animal models, endocannabinoid release in the magnocellular hypothalamus modulates glutamatergic and gamma-aminobutyric acid (GABA)ergic inputs to oxytocin neurons that tune their burst firing during parturition and lactation (33). At the periphery, endocannabinoid signaling was placed as a key node of placental autonomy and a trigger for trophoblast invasion (34). Likewise, the suckling reflex, one of the first perinatal functions to ensure the individual’s survival, is shaped by type 1 cannabinoid receptor (CB<sub>1</sub>R)-dependent signaling pathways (35), and its disruption experimentally by CB<sub>1</sub>R antagonists provokes death.

Neuropsychiatric disorders represent a significant section of human illnesses in Western societies. The longitudinal Ottawa Prenatal Prospective Study and the Maternal Health Practices and Child Development Study showed that children of both low-risk (Ottawa Prenatal Prospective Study) and high-risk (Maternal Health Practices and Child Development Study) pregnant women exhibit signs of neuropsychiatric disturbances at later ages. In this review, we collected scientifically substantiated information showing that prenatal, perinatal, or adolescent cannabis exposure can interfere with brain

ontogeny, inducing subtle and long-lasting neurofunctional impairments.

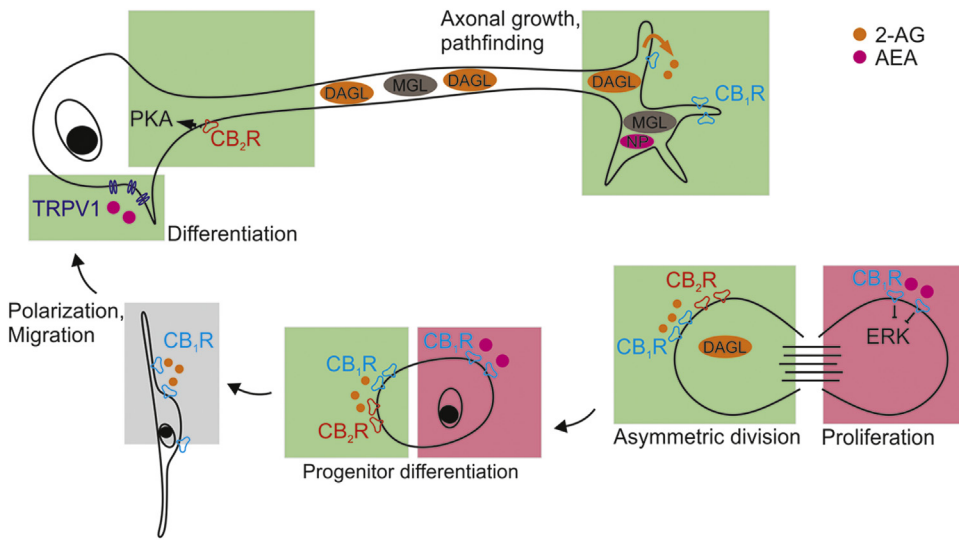
## ENDOCANNABINOID SIGNALING IS A SUBSTRATE OF CANNABIS IN FETAL CNS

Initial understanding about how cannabinoid ligands exert their cellular actions was based on observations in the adult nervous system. This cross-correlational landscape has changed recently, with mechanistic analysis in embryonic brains and peripheral tissues dissecting the mode of action for THC and other CB<sub>1</sub>R receptor ligands (36–38). In fact, by consensus, CB<sub>1</sub>R is the major neuronal target of THC in both the adult and embryonic brain (27,36,39,40). Yet, signaling via type 2 cannabinoid receptors (CB<sub>2</sub>R) (41), G-protein coupled receptor 55 (42), peroxisome proliferator-activated receptors (43), and transient receptor potential ion and cation channels (TRPM8, TRPA1, TRPV2, and probably also TRPV1) (44) has also been described (42,45). In particular, CB<sub>2</sub>R (46) and TRPV1 (47) signaling may be relevant for neuronal development for their involvement in the control of neural progenitor proliferation (40) and neurite outgrowth and directional guidance (Figure 1).

The molecular identification of cannabinoid-sensing receptors prompted the exploration of endogenous ligands, which are lipophilic derivatives of arachidonic acid: N-arachidonylethanolamide (anandamide [AEA]) (48) and 2-arachidonoylglycerol (2-AG) (49,50). In the adult brain, presynaptic CB<sub>1</sub>Rs (51–54) produce state-dependent, bidirectional modulation of synaptic neurotransmission at both inhibitory and excitatory synapses (55). Significantly, endocannabinoids affect both short-term and long-term synaptic plasticity, and as a general rule, attenuate presynaptic neurotransmitter release. Disturbance of CB<sub>1</sub>R-mediated control of synaptic plasticity is typically seen upon drug exposure and likely leads to neuronal circuit failures (56–59). Besides, CB<sub>1</sub>Rs might also be found along the somatodendritic plasma membrane of neurons (60). Yet, the study of their trafficking, particularly endocytosis, suggests vastly different constitutive cycling and limited ligand binding.

Cannabis is clearly not THC alone. Instead, the large majority of plant components, whose relative composition depends on the plant variety (e.g., selectively cultivated subspecies), do not directly interact with CB<sub>1</sub>Rs. Such CB<sub>1</sub>R-independent, or more generally receptor independent, actions underpin the importance of distinguishing between different varieties of cannabis when describing their psychotropic and medicinal actions. Most plant cannabinoids investigated so far interact with TRPV1, TRPV2, TRPM8, and TRPA1 channels (61). The propyl analogue of THC,  $\Delta^9$ -tetrahydrocannabivarin, a minor cannabinoid, is a neutral antagonist for CB<sub>1</sub>R (62) and/or can weakly inhibit 2-AG biosynthesis (61). Similarly, the other most abundant cannabinoid, cannabidiol, has several non-CB<sub>1</sub>R targets (63), including its inhibition of endocannabinoid inactivation (64). This, though indirectly, can augment CB<sub>1</sub>R activity.

Understanding the role of endocannabinoid signaling in CNS ontogeny reached a critical advance when not only cannabinoid receptors (65) but also key nodes of the enzymatic machinery that controls endocannabinoid bioavailability were explored in the developing brain (66–68). Accordingly,  $\alpha$  and  $\beta$  isoforms of *sn*-1-diacylglycerol lipases and *N*-acyl-phosphatidylethanolamine-selective phospholipase D generate 2-AG and AEA, respectively



**Figure 1.** Ligands, enzymes, and receptors of the endocannabinoid system and their assumed roles in developmental neurobiology. Anandamide (AEA) inhibits neural progenitor proliferation by blocking extracellular signal-regulated kinase (ERK) and differentiation through type 1 cannabinoid receptors (CB<sub>1</sub>R) (78). In turn, 2-arachidonoylglycerol (2-AG) (188) or the pharmacologic activation of CB<sub>1</sub>R (76,189) or type 2 cannabinoid receptors (CB<sub>2</sub>R) (190) promotes these actions. Polarization and migration of neurons involves CB<sub>1</sub>R signaling and a mixture of autocrine and paracrine mechanisms. Directional axonal growth is triggered by TRPV1, CB<sub>1</sub>R, and CB<sub>2</sub>R. Note that the molecular architecture of endocannabinoid metabolism in developing neurons is different from those in adults with both synthesis and degrading activities accumulating in the same

cell yet at distinct subcellular foci (191). DAGL, diacylglycerol lipase; MGL, monoacylglycerol lipase; NP, *N*-acyl-phosphatidylethanolamine-selective phospholipase D; PKA, protein kinase A (43); TRPV1, transient receptor potential cation channel subfamily V, member 1.

(69,70). In contrast, monoacylglycerol lipase and fatty acid amide hydrolase inactivate 2-AG and AEA, respectively (71–74). In the adult brain, this enzymatic machinery is linked to mature synaptic sites to acutely regulate synaptic transmission via the on-demand synthesis (and degradation) of endocannabinoids (59,75). In the developing brain, endocannabinoid levels are regulated by a molecularly similar machinery that contributes to the control of neural proliferation, survival, lineage commitment (40,76–78), and directional axonal growth (36,71,79,80). Apart from these physiological functions of endocannabinoid signaling (Figure 1) and more in the direction of neuropsychiatric disorders, research on CB<sub>1</sub>R and CB<sub>2</sub>R soon shifted onto brain territories and receptor systems that are directly linked to neurological diseases or behavioral functions. This was a critical step in clinical cannabinoid research: while basic knowledge about the role of the endocannabinoid system in neurodevelopment was primarily acquired principally in the cerebral cortex (40,71,80,81), these focused studies generated data in circuitries more implicit to specific pathologies.

The series of discoveries collated here involved, besides some key human studies, elaborate animal experiments. In most cases, purified THC or potent cannabinoid receptor agonists/antagonists were applied at doses higher than those usually taken by humans. We are aware that extrapolation of experimental data to human pathobiology might raise concerns. Therefore, we only emphasized points that are coincidentally supported by both lines of evidence, and contextualized drug action and outcome bearing in mind differences in dose, exposure time, gender, and genetic heterogeneity.

### DISPARATE OUTPUT OF THE DOPAMINERGIC MESOLIMBIC SYSTEM UNDERSCORES BEHAVIORAL ABNORMALITIES, MOOD DISORDERS, AND DEPRESSION IN THC-EXPOSED OFFSPRING

Brain regions in rodents, primates, and humans responsible for mood and conscious behaviors are generally referred to as

limbic regions. These areas harbor either dopaminergic neurons or their D<sub>1</sub>, D<sub>2</sub> dopamine receptor (D<sub>1</sub>/D<sub>2</sub>R)-containing efferents and postsynaptic targets, underpinning dopamine-mediated connectivity. Early animal studies indicated that prenatal exposure to hashish containing a mixture of THC (11.8%), cannabiol (5.7%), and cannabidiol (9.7%) (at 20 mg/kg) throughout gestation gender-dependently affects the dopaminergic system in the limbic forebrain of the offspring—weeks after the actual exposure (82): in female rats, a transient decrease of D<sub>1</sub>R appeared by the second postnatal week and was accompanied by a decreased level of dopamine and its metabolites. These changes were transient and normalized by the end of the first postnatal month. In male rats, the activity of tyrosine hydroxylase, the rate limiting enzyme of dopamine synthesis, and dopamine metabolite levels increased by the end of the first postnatal month, leaving receptor numbers unaltered (82). Neurotransmitters, present from early stages of brain development, exert trophic effects on both target neurons and the maturation of afferent inputs (e.g., during the last prenatal and first three postnatal weeks in rodents), producing a critical time window for drug action (83). THC-induced reorganization of the dopamine system occurs within this sensitive period and might disrupt reward circuits by genetic and epigenetic modifications (84,85). Studying striatal dopamine and opioid-related genes in human fetal subjects exposed to marijuana and in a possible corresponding animal model of prenatal THC exposure, the nucleus accumbens of adult THC-exposed offspring showed increased 2meH3K9 repressive mark and decreased 3meH3K4 on and RNA polymerase II at the *Drd2* locus (coding for D<sub>2</sub>R) (85). In adults, acute marijuana exposure induced alterations in mesolimbic dopaminergic activity that underlie euphoric, motivational, and emotional imbalances (86). Experiments in rats showed that this effect is due to increased neuronal firing (87) and heightened tissue dopamine levels (88). Indeed, adult male rats perinatally exposed to THC

display [dosage as in (82)] changes in sociosexual approach behavior, in parallel with an imbalance in dopaminergic neuron activity. This reflects the importance of a potential marijuana-induced dopamine imbalance in limbic circuits (89).

Despite early studies being available (90,91), our understanding of how THC affects human neuropsychiatric disorders experienced a quantum leap when the presence and distribution pattern of CB<sub>1</sub>Rs were identified in the midgestational fetal human forebrain and found concentrated in the amygdala and hippocampus (92). Using *in situ* hybridization to visualize messenger RNA (mRNA) distribution in human midgestational fetuses (weeks 18–22), decreased D<sub>2</sub>R mRNA expression was shown in the basal amygdala. This change correlated with the amount of maternal marijuana intake, ranging from 0 to >.89 average daily joints during pregnancy. This change was specific, since no similar alteration was detected in the hippocampus or striatum, the latter being a key output target of the dopaminergic nigrostriatal tract (93). Male, but not female, subjects exhibited significant D<sub>2</sub>R loss in human fetuses, which corroborates gender-related differences in animal studies (82,89). This gender-specific imbalance in dopaminergic development might underscore that boys exhibit greater vulnerability when tested for performance in attention, learning, and memory in association with *in utero* marijuana exposure (94–96). Since the structures involved are critical for the development of behavioral and mood disorders (97), a shift in dopamine receptor expression could contribute to depressive symptoms (98) and impaired social behaviors, as reported in children upon longitudinal follow-up (11,94).

Investigations about the possible incidence of prenatal marijuana exposure on child depressive symptoms at 10 years of age showed unexpected results. Based on the Maternal Health Practices and Child Development Project and using the Children's Depression Inventory, a self-report measure of childhood depression, repeated maternal marijuana use in the first and third semesters was significantly associated with the Children's Depression Inventory score (98). Most women cease or minimize drug abuse once becoming aware of their pregnancies (99). Nevertheless, expanded analyses of the effects by taking individual trimesters of marijuana exposure as variables revealed that depressive symptoms in children in fact result from maternal cannabis consumption during the first trimester (98). The exact cellular and system underpinnings of this phenomenon need further clarification since histochemical and biochemical data from first trimester (early) fetuses are currently unavailable.

Continued perinatal marijuana exposure affects dopamine signaling beyond the mesolimbic system. Of note is the nigrostriatal circuit where decreased tyrosine hydroxylase activity is coupled with increased receptor numbers in the striatum, as shown in rat offspring (82). This indicates either that THC-induced presynaptic hypoactivity triggers an upregulation of postsynaptic D<sub>1</sub>R/D<sub>2</sub>Rs or that activation of presynaptic D<sub>2</sub>Rs alters neurotransmitter synthesis and release. Since the striatum is a major subcortical station in extrapyramidal movement modulation, it is not entirely unexpected that motor activity is impaired in a rat model of prenatal marijuana administration (100,101). Nevertheless, no direct evidence for a shift in nonmesolimbic dopamine systems was identified in the human brain. No changes in dopamine

receptor mRNA expression were shown in the striatum of human fetuses prenatally exposed to marijuana, either (93). Moreover, longitudinal human studies do not associate an increased risk of neurological deficits after maternal cannabis use with motor impairment, typically Parkinson's disease. The indirect involvement of the endocannabinoid system in evoking neurological malfunction in the offspring, nevertheless, remains plausible since maternal marijuana use impairs opioid gene expression in the fetal caudate putamen (93). While the endocannabinoid and opioid systems likely interact (102) (and below), neither the possibility nor the mechanism of a possible indirect THC-dependent mechanism has been dissected.

Despite a combination of human and experimental studies pointing to cannabis-induced deficiencies in the developmental organization of the dopamine system, critical questions as to the molecular mechanism of cannabis action remain open. As such, the presence of CB<sub>1</sub>Rs—and more so of CB<sub>2</sub>Rs—on dopaminergic neurons remains contentious. Selective CB<sub>1</sub>R antagonism (by arachidonylcyclopropylamide) limits memory retention in a D<sub>1</sub>R-dependent manner in the basolateral amygdala in male mice (103). Secondly, the firing of midbrain dopamine neurons in rats is also modulated by CB<sub>2</sub>R agonists (104). Thus, understanding receptor identity, temporal control of expression, and functional significance over critical developmental time windows will be imperative to dissect key cellular changes that persist after early cannabis exposure and contribute to lasting deficits in the organization and function of the corticolimbic dopamine system.

### PRENATAL INTERACTION OF OPIOID AND ENDOCANNABINOID SYSTEMS: IMPLICATIONS FOR NEUROPSYCHIATRIC DISORDERS DURING POSTNATAL LIFE

The opioid system consists of neurons that harbor the enzymatic pathways to generate endogenous opioid peptides and/or cognate receptor systems in the central and peripheral nervous systems. Opioids influence nociception, motor control, the regulation of emotions, reinforcement, and cognition (105). Endogenous opioid receptor agonists include enkephalins and endorphins, which trigger reinforcement (the strengthening of a subsequent behavior upon a specific stimulus) via mu and delta opioid receptors. Moreover, dynorphins mediate aversion (repugnance to a certain previously experienced stimulus) and dysphoria (profound state of unease) via kappa opioid receptors (106). These receptors, nevertheless, can be equally activated by exogenous opiates, which—like cannabis—are exploited both as medication (morphine, codeine) and as drugs of abuse (heroin).

Neurons in limbic regions are simultaneously targeted by the endocannabinoid and opioid systems, giving rise to coordinated and cross-dependent mechanisms in defining cellular actions and output (102,107). The interaction between the endocannabinoid and opioid systems has been elaborated experimentally and points to the modulation of behavioral responses linked to drug reinforcement, reward, or relapse (108,109). Enkephalin and beta-endorphin release is stimulated by endocannabinoids in both the nucleus accumbens (110) and ventral tegmental area (111), the latter being the origin of mesocorticolimbic dopamine projections. These



interactions manifest, at the behavioral level in CB<sub>1</sub>R<sup>-/-</sup> mice and drug-treated rats, in reduced addictive effects of opiates (112), failure to self-administer morphine (113), and reduced heroin seeking upon CB<sub>1</sub>R antagonism (114).

Human investigations revealed that maternal marijuana use affects fetal expression of opioid-related genes. Using a multiple regression paradigm for confounding variables that included alcohol and cigarette use, prenatal marijuana exposure was shown to significantly alter opioid receptor expression in the mesolimbic forebrain: increased mu and kappa receptor levels were found in the amygdala and mediodorsal thalamic nucleus, respectively (115). These observations are strengthened by prenatal THC (.15 mg/kg, daily) induced reduction of preproenkephalin mRNA expression in the rat nucleus accumbens (84) during early development.

The dependence of opioid-mediated mechanisms on endocannabinoids triggered developmental investigations to test whether prenatal marijuana exposure could impair the opioid system in affected offspring and underscore behavioral deficits later in life. Studies in both animals and humans suggest that these effects can indeed occur. In adult rats prenatally exposed to THC, preproenkephalin mRNA expression is elevated in the nucleus accumbens and the central and medial amygdala (84). The selective alteration of the preproenkephalin gene is intriguing since enkephalin is well known to modulate hedonic states (116,117). This neuroanatomical finding is coupled with behavioral changes in adulthood: while animals show similar heroin intake in a self-reinforcement paradigm, they exhibit shorter latency to the first active lever press, respond more for low heroin doses, and have higher heroin seeking during mild stress and drug extinction (84). Drug abstinence, especially during its early phase, is a very stressful event for drug-dependent subjects. The increase of heroin seeking in THC-exposed offspring might reflect a behavioral response to stress, which intensifies the motivation for drug use. In humans, disrupted limbic organization in marijuana-exposed fetuses, including opioid receptor and D<sub>2</sub>R changes in the mediodorsal thalamus and/or amygdala, respectively, suggests susceptibility for neuropsychiatric impairments in later life. Indeed, longitudinal human studies show that prenatal marijuana-exposed children and young adults exhibit deficits in executive function and academic achievement (11,118) and also frequently suffer from depression, anxiety, inattention, delinquency, and psychosis (22,96,119).

Besides opioids, marijuana is often co-abused with other drugs (120), which can either directly impact the same neurotransmitter system as cannabis or produce complex outcomes through actions on additional brain circuits. As such, alcohol and cocaine are the most frequently co-abused substances (120,121). Animal studies in adults offer insights at molecular interactions between cannabinoids and alcohol since chronic ethanol intake increases limbic endocannabinoid levels (122) and reduced CB<sub>1</sub>R gene expression in hippocampus, striatum, and ventral hypothalamus (123). At the behavioral level, cannabinoid agonists/antagonists reduce ethanol intake, self-administration, and seeking (124). For the developing brain, fetal alcohol syndrome is the most devastating outcome of chronic alcohol exposure in utero. Through data from fish and rodent models, we conclude that CB<sub>1</sub>R expression is reduced by ethanol, in part by microRNA regulation (125), and

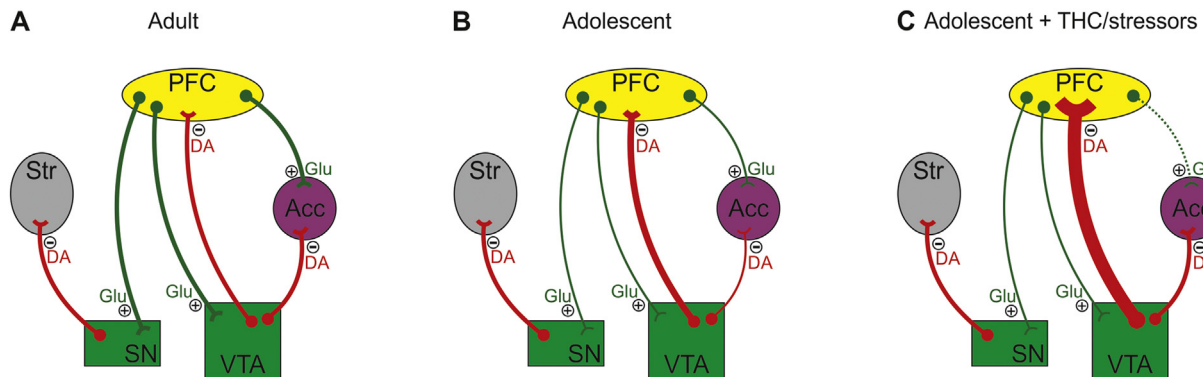
perturbed neurodevelopment (126,127) is, in part, due to defunct signaling at CB<sub>1</sub>Rs (126,128). Nevertheless, whether alcohol and marijuana interact during pregnancy remains controversial. Some human longitudinal studies on Caucasian and African-American women suggest that prenatal marijuana and alcohol are independent predictors of academic child performance (96,118). Yet another study warns that individual effects of substance use, particularly of subtle ones during pregnancy, might not be detected in smaller populations, precluding interaction analysis (129). Similarly, cocaine is co-abused with marijuana (up to 44% in specific socioeconomic cohorts) (121), but longitudinal follow-up of affected offspring is insufficient to justify the interaction of these substances.

### **SCHIZOPHRENIA: DEVELOPMENTALLY REGULATED DYSBALANCE OF EXCITATION AND INHIBITION DUE TO ALTERED ENDOCANNABINOID SIGNALING?**

Schizophrenia is a chronic and devastating mental disorder that typically presents in early adulthood (130). The terminology defines a heterogeneous group of imperfectly understood brain disorders characterized by alterations in higher functions related to perception, cognition, communication, planning, and motivation (131). Cognitive impairments are considered to be the core feature of the illness and develop upon genetic predisposition and/or environmental challenges (132).

The complexity of positive (hallucinations, delusions, lack of insight), negative (poverty of thought, anhedonia, apathy, reduction in social life, and affective expression), and cognitive (inability to sustain attention, loss of working/short-term memory) symptoms are key reflections of disrupted high-order brain functions, which are typically associated to the prefrontal cortex. Final development of the prefrontal cortex is delayed until adolescence, thus being one of the remarkably late-maturing cortical areas. Although difficulties exist to delineate its precise time window, adolescence broadly covers the period between the nonreproductive and reproductive stages in humans between 11 and 18 years of age (133). For experimental purposes, a similar developmental window spanning postnatal days 36 to 48 was suggested for rodents (134). A causal role of the prefrontal cortex to gaining ability to plan, maintain information online (working memory), solve complex cognitive tasks, and undertake self-regulation is suggested by the rapid unfolding of these skills during early adulthood (135).

One of the neuroanatomical substrates of the functional changes in schizophrenia includes the reorganization of the dopamine system (Figure 2) (133). Animal studies show that the balance between mesocortical (prefrontal cortex targeting) and mesolimbic (nucleus accumbens targeting) dopamine outputs of the substantia nigra and ventral tegmental area significantly shift toward a greater dominance of cortical dopamine in early adolescence: tissue dopamine concentrations, as well as afferentation density, increase in the prefrontal cortex of adolescent rodents (136) and primates (137). These changes are paralleled by the ontogenic disappearance of an early dopamine autoreceptor-like modulation of dopamine synthesis, which likely inhibits dopamine synthesis before adolescence (138), and the pruning (refinement) of afferent projections (139).



**Figure 2.** Major dopaminergic pathways of the brain. In adolescence, mesocortical dopamine (DA) influence peaks in the prefrontal cortex (PFC) but dopamine activity becomes lower in the nucleus accumbens (Acc). The increased inhibition of PFC pyramidal cells results in a decreased excitatory glutamatergic output onto the subcortex, further amplifying the increased inhibitory dopaminergic tone on the PFC. Compared with nigrostriatal input, mesolimbic but especially mesocortical afferents are sensitive to environmental stressors/drug abuse that may escalate the dopamine imbalance between the cortical and subcortical integration centers in adolescent marijuana abuse. Altered line thickness across the named conditions denotes changes in strength of expression/effects. Glu, glutamate; SN, substantia nigra; Str, striatum; THC,  $\Delta^9$ -tetrahydrocannabinol; VTA, ventral tegmental area.

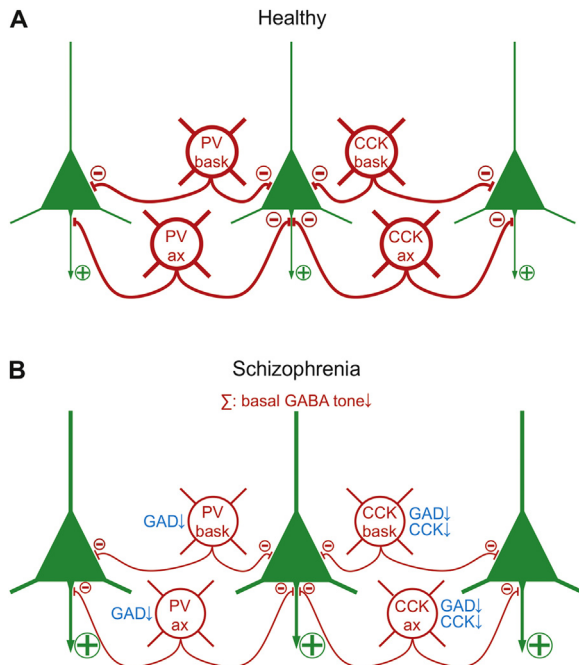
Another important aspect of adolescent brain development is the rewiring of the prefrontal cortex via the modulation of local interneurons (Figure 3). Interneurons encompass different subtypes of inhibitory cells with locally targeting axons, which enable them to gate circuit output through the entrainment of large assemblies of glutamatergic (output) neurons (140). Most notably, parvalbumin-containing and cholecystokinin (CCK)-containing basket and axo-axonic interneurons typically exert synchronous population control through perisomatic inhibition of pyramidal cells (140). In the adolescent prefrontal cortex, new and subtype-specific GABAergic inputs form on pyramidal cells (141). Axo-axonic cells display reduced synaptic inputs onto superficial pyramidal cells, which are more markedly involved in schizophrenia (both their presynaptic and postsynaptic GABA markers change significantly compared with deep layer principal cells) (141). Further, parvalbumin-containing interneurons exhibit delayed maturation and vulnerability to altered redox states typical for schizophrenia, enhancing deleterious insults on inhibitory circuit establishment (142).

The age-dependent overlap of prefrontal cortex maturation and the highest prevalence of marijuana consumption in teenagehood prompt the question whether continued exposure to THC mechanistically underscores the pathogenesis of schizophrenia. A major driving force behind this hypothesis is that endocannabinoids are acutely involved in the depolarization-induced suppression of synaptic activity, which, if disturbed, could impinge upon activity-driven synapse development. The postulate that THC disrupts the physiological control of endocannabinoids over glutamate and GABA release and affects adolescent experience-dependent maturation of neural circuitries in the prefrontal cortex (131) is supported by longitudinal prospective studies in Swedish conscripts and in a New Zealand birth cohort, which uncovered that adolescent marijuana use increases the likelihood of schizophrenic symptoms in adulthood (143), an effect that is unrelated to pre-existing psychosis (144). There is a significantly greater risk in early users (<15 years), which positively correlates with the frequency and potency of marijuana used in British, Swedish, Dutch, and New Zealand

cohorts (22,144–146). However, increased risk might rely on coincident genetic predisposition, e.g., polymorphism of catechol-*O*-methyltransferase, a major dopamine-degrading enzyme (147).

Several further studies suggest the late developmental etiology (including the reduced protective action of the endocannabinoid system during a vulnerable period), pathogenesis (impaired neurotransmitter release), and pathophysiology (disrupted network control by altered prefrontal cortex connectivity) of schizophrenia-like symptoms in subjects with a history of cannabis consumption during adolescence. Significant alterations occur in schizophrenia subjects within parvalbumin and CCK interneurons: human pathology studies highlight that both CCK mRNA and protein levels are reduced in the prefrontal cortex of schizophrenia subjects (148). These observations are supported experimentally since parvalbumin-containing interneurons contain less glutamic acid decarboxylase 67 in models of schizophrenia. Accordingly, THC exposure (doses ranging from 2.5 mg/kg to 10 mg/kg) between postnatal days 35 and 45 (adolescence) in rodents reduces glutamic acid decarboxylase 67 expression in both parvalbumin and CCK-containing interneurons coincident with decreased basal GABA levels within the prefrontal cortex, which perpetuate negative symptoms and cognitive signs (149). Defunct inhibition might lead to runaway excitation as suggested by increased glutamate content in the prefrontal cortex (149) and particularly in the anterior cingulate cortex of adolescent chronic marijuana smokers (150).

A large genome-wide association study identified >100 loci of single nucleotide polymorphisms associated with schizophrenia (151). Notably, polymorphisms in the gene encoding the CB<sub>1</sub>R (*Cnr1*) but not *Faah* may confer susceptibility to hebephrenic schizophrenia (152,153), as well as psychotomimetic effects at commencement of cannabis use (154). Moreover, the genotype for Akt1 (*v-Akt* murine thymoma viral oncogene homolog 1), a kinase determinant of growth-factor induced neuronal survival and CB<sub>1</sub>R-mediated neurite outgrowth (155), was shown to underlie the risk of psychosis in cannabis users aged 18 to 65 years (156).



**Figure 3.** Schema of inhibitory wiring of principal cells and its modifications in the prefrontal cortex of schizophrenics. **(A)** Parvalbumin<sup>+</sup> and CCK<sup>+</sup> interneurons inhibit the activity of pyramidal cells by innervating their perisomatic and axon initial segments, thus synchronizing population discharges. **(B)** In schizophrenia, glutamic acid decarboxylase (GAD) expression in parvalbumin<sup>+</sup> and CCK<sup>+</sup> interneurons decreases, dampening inhibition and, conversely, enhancing excitability and desynchronization of pyramidal cell assemblies in the prefrontal cortex. ax, axo-axonic cell; bask, basket cell; CCK, cholecystokinin; GABA,  $\gamma$ -aminobutyric acid; PV, parvalbumin.

Even though these data argue for a genetically coded predominance of the illness, the delayed onset of prefrontal cortex maturation and its changes in relation to marijuana render the term “developmental disorder” relative and prenatal drug effects on delayed disease manifestation largely ambiguous and speculative. Although we have seen that prenatal marijuana exposure leads to significant alterations in the dopamine system, a causative link or effect to prefrontal cortex development remains elusive at both the clinical and experimental levels. It is tempting to speculate that the dopamine imbalance hypothesis [positive or negative symptoms of schizophrenia are related to excessive or low dopamine activity, respectively (157)] might be due to changes in dopamine receptor expression evoked by continued maternal THC use. Nevertheless, prenatal studies offer mechanistic insights only on how cannabinoids affect dopamine network maturation. In contrast, the key question whether THC or other CB<sub>1</sub>R agonists displace endocannabinoids to establish causality to disease manifestation is still unknown. Similarly, and in cohesion with the neurodevelopmental hypothesis, we can neither confirm nor exclude that the disturbed migration and differentiation of GABAergic interneurons due to impaired endocannabinoid signaling (158) could be a structural substrate of the subtle reorganization of neuronal circuitries, thus sensitizing the brain to schizophrenia.

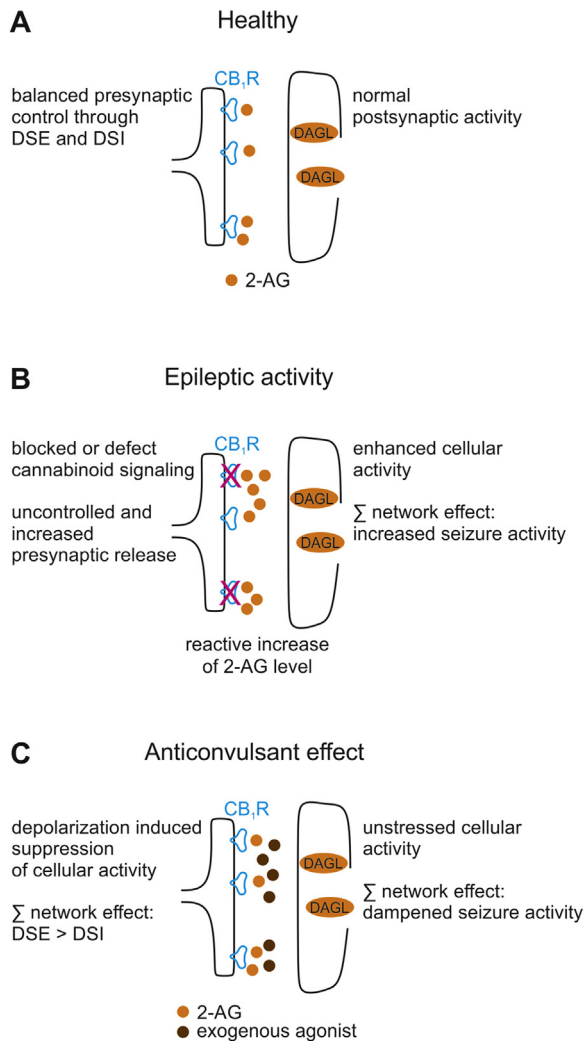
Collectively, and to the best of our knowledge, longitudinal or cohort studies that would indicate an increased risk of schizophrenia related to maternal marijuana use remain elusive, even though marijuana abuse is a predictor for an earlier onset of schizophrenia in patients with nonfamilial schizophrenia, especially in the presence of predisposing genetic factors (159). Among the different pieces of a manifold puzzle, only adolescent but not prenatal THC exposure has so far been identified as environmental predisposition precipitating schizophrenia (160,161).

### EPILEPSY AND THE JANUS-FACED GABA SIGNALING OF THE DEVELOPING BRAIN

Progression of synchronous population discharges leads to epileptic activity (Figure 4), which is effectively prevented and controlled by the recruitment of GABAergic inhibition (162). Given the efficacious modulation of synaptic neurotransmission by endocannabinoids, plant-derived cannabinoids emerge as medically relevant antiepileptic/anticonvulsive compounds (Figure 4) (163–167). Nevertheless, THC and GABA actions during development largely differ from those in the adult brain, and their impact cannot be interpreted on premises specific to the adult nervous system.

While GABA is widely known to be the major inhibitory neurotransmitter in the adult brain, it is excitatory in most brain structures during early development (168) and acts in synergy with glutamate to produce early-life network activity (169). Later (e.g., neonatal hippocampus) GABA signaling sets the threshold for circuit excitability (170) and is already sensitive to CB<sub>1</sub>R modulation. Using brains from first postnatal week rats, Bernard *et al.* (171) showed that interfering with signaling at CB<sub>1</sub>Rs results in pathological activity *in vitro* and *in vivo*: abnormal enhancement or impairment of CB<sub>1</sub>R activity led to the cessation of neuronal activity or epileptic hyperexcitability, respectively. Since the first postnatal week in rodents corresponds to the third trimester of gestation in humans in terms of brain development and physiological activity (172), persistent maternal marijuana use is predicted to be harmful in human fetuses, as well. Notably, prenatal exposure to the CB<sub>1</sub>R agonist WIN55,212-2 accelerates decays in synaptic plasticity in hippocampal slices and decreases basal and potassium-evoked extracellular glutamate levels in the hippocampus of juvenile and adult rats (173). It must be emphasized, however, that while WIN55,212-2 is a full and potent agonist at CB<sub>1</sub>Rs, THC is only a partial agonist, and that animal studies have the tendency to use high to very-high doses of this and similar compounds. Thus, experimental research might not mimic accurately the actual intake of THC during gestation through marijuana use. Notably, however, cannabidiol is being indicated as a relatively safe antiepileptic predominantly acting in a CB<sub>1</sub>R-independent fashion.

Prenatal drug or toxin exposure can lead to seizures later in life. Crude toxins, like methyl-mercury, can lead to severe epilepsy in both animal (174) and human (175) offspring. Opiates, such as morphine, are frequently abused during pregnancy (176), particularly in high-risk urban populations (177), and prenatal morphine exposure enhances seizure susceptibility in the limbic system of adult male rats (178).



**Figure 4.** The possible biological roles of the type 1 cannabinoid receptor (CB<sub>1</sub>R)/endocannabinoid system in the control of epileptogenic activity. Physiologically **(A)**, the retrograde messenger 2-arachidonoylglycerol (2-AG) controls presynaptic neurotransmitter release via CB<sub>1</sub>Rs. **(B)** Blocked or defect endocannabinoid signaling enhances presynaptic activity, which provokes compensatory 2-AG production. **(C)** Exogenous CB<sub>1</sub>R agonists can suppress presynaptic activity, especially at excitatory synapses, which might dampen seizure severity. DAGL, diacylglycerol lipase; DSE, depolarization-induced suppression of excitation; DSI, depolarization-induced suppression of inhibition.

Similarly, in utero exposure to cocaine leads to increased seizure susceptibility in adult rat offspring (179,180). Although both opioid and cocaine exposure can influence endocannabinoid signaling, we are unaware of any data being available to confirm cannabinoid receptor involvement in the above mentioned mechanisms, even though the selective dopamine transporter inhibitor 3beta-(4-methylphenyl)-2beta-[3-(4-chlorophenyl)isoxazol-5-yl]tropane was suggested to block cocaine-induced locomotor stimulation via the positive allosteric modulation of CB<sub>1</sub>Rs (181). While a direct link between prenatal or perinatal marijuana exposure and the development of epilepsy later in life has not been highlighted in previous

longitudinal human or in experimental animal studies, the use of cannabidiol-enriched cannabis in children with treatment-resistant epilepsy led to unexpected results (182). Over 80% of the parents reported a reduction in their child's seizure frequency while taking cannabidiol-enriched cannabis with mild side effects including drowsiness and fatigue (182). Of note, 13 of the included children were diagnosed with Dravet syndrome, a rare and treatment-resistant form of epilepsy. The anticonvulsive effects of cannabidiol were also confirmed in adult patients suffering from refractory secondarily generalized epilepsy (183). Cannabidiol's reported benefits are likely due to its antioxidant and anti-inflammatory properties, as well as its enhancement of endocannabinoid retention to dampen hyperexcitability (25,184). Even though cannabidiol is often co-administered with THC to protect against THC's harmful effects, direct experimental evidence on a molecular pathway by which cannabidiol counter-regulates THC effects to maintain a benign therapeutic window is lacking. Thus, it is plausible that distinct types of cannabis preparations, often derived from different varieties of the plant, might impact the same biological phenomenon (e.g., pathological condition) in a different manner and to substantially different extents.

## CONCLUSIONS

Penetrating any developing neural system with external stimuli leads to functional alterations. The endocannabinoid system is an evolutionarily conserved signaling network that guides critical aspects of brain development (185). In this review, we highlighted human and rodent data to show that prenatal exposure to CB<sub>1</sub>R agonists impacts neuronal development, leading to altered neurotransmitter and neuronal circuit settings. While ensuing neuroanatomical changes and behavioral consequences in the offspring are evident, the intriguing question remains why some neuropsychiatric diseases evoked by adult or adolescent marijuana consumption do not manifest in offspring with prenatal drug exposure. The quasi-absence of epileptiform activities or schizophrenia symptoms in children with maternal cannabis use does not only highlight differences in endocannabinoid function in adult versus the fetal brain but demonstrates the need for further mechanistic studies to dissect molecular and cellular determinants of cannabinoid action.

This review discussed data from basic and clinical neuroscience in relation to cannabis use and brain development. Nevertheless, the impact of cannabis use on the dependence/use of drugs considered more harmful later in life was only briefly touched upon here. Almost all of those who tried cocaine and heroin first used alcohol, tobacco, and cannabis (186), and regular cannabis users are most likely to later use heroin and cocaine (187) with an earlier age of cannabis use onset being a further risk factor (186). Thus, social complexity specifics must be considered when concluding on the actual danger of cannabis use for the development of neuropsychiatric disorders.

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