Review

Biological Psychiatry

Maternal Immune Activation and the Endocannabinoid System: Focus on Two-Hit Models of Schizophrenia

Michele Santoni and Marco Pistis

ABSTRACT

The devastating effects of the COVID-19 pandemic have underscored the significant threat that infectious diseases pose to our society. Pregnancy represents a period of heightened vulnerability to infections, which can compromise maternal health and increase the risk of neurodevelopmental disorders in offspring. Preclinical and clinical investigations suggest a potential association between maternal immune activation (MIA), which is triggered by viral or bacterial infections, and increased risk for neurodevelopmental disorders such as autism and schizophrenia. Genetic and environmental factors may contribute to the overall risk. Therefore, the two-hit hypothesis of schizophrenia suggests that MIA could act as a first trigger, with subsequent factors, such as stress or drug abuse, exacerbating latent abnormalities. A growing body of research is focused on the interaction between MIA and cannabis use during adolescence, considering the role of the endocannabinoid (eCB) system in neurodevelopment and in neurodevelopmental disorders. The eCB system, crucial for fetal brain development, may be disrupted by MIA, leading to adverse outcomes in adulthood. Recent research indicates the eCB system's significant role in the pathophysiology of neurodevelopmental disorders in preclinical models. However, findings on adolescent cannabinoid exposure in MIA-exposed animals have revealed unexpected complexities, with several studies failing to support the exacerbation of MIA-related abnormalities. In this review, we delve into the functional implications of the eCB system in MIA models, emphasizing the role of 2-AG (2-arachidonoylglycerol) signaling in synaptic plasticity and neuroinflammation and its relevance to the two-hit model of schizophrenia.

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Infectious diseases pose a significant threat to our society, as underscored by the detrimental impact of the COVID-19 pandemic. Pregnancy is a period of heightened susceptibility to infections, which pose risks to maternal health and increase the likelihood of neurodevelopmental disorders in offspring (1–3). Preclinical and clinical investigations suggest a link between maternal immune activation (MIA), which is triggered by viral or bacterial infections, and the onset of neurodevelopmental disorders such as autism and schizophrenia (4,5). Numerous epidemiological studies have emphasized the association between an imbalance in pro- and anti-inflammatory cytokine levels during pregnancy and a heightened risk of psychosis in later life (4,6). Animal models focused on MIA have demonstrated that perinatal disturbances can result in detrimental effects on offspring (7–10). These detrimental outcomes have been observed in various mammalian species, including mice, rats, and nonhuman primates (11,12). Early research used prenatal exposure to live pathogens, such as the influenza virus (13–15). Using live pathogens in models is useful for establishing a causal link between the pathogen and offspring outcomes but necessitates technical precautions. Two main immunogenic approaches have been developed to overcome these challenges: administration of lipopolysaccharide or poly(I:C) (polyinosinic:polycytidylic acid) to pregnant rodents. Lipopolysaccharide, a cell wall component of gram-negative bacteria, stimulates an innate immune response via the toll-like receptor 4 (TLR4) pathway (16). Another MIA model is based on poly(I:C), a double-stranded synthetic RNA that mimics a viral infection by triggering an innate immune response (17–19). Poly(I:C) binds to TLR3, triggering a viral-like acute inflammatory response and resulting in the synthesis of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, tumor necrosis factor α (TNF-α), and interferons (IFNs) (20). Offspring of MIA-exposed dams display a multitude of impairments, including deficits in sensorimotor gating, social interaction, working memory, and behavioral despair (16,21,22). Prenatal insults in rodents lead to abnormal neurodevelopmental paths, disrupting postnatal brain maturation and potentially causing behavioral abnormalities (22,23). MIA may serve as a primer, heightening the offspring's vulnerability to environmental insults later in life. The two-hit hypothesis of schizophrenia, proposed by Bayer *et al.* (24), posits that genetic risk factors act as the first hit, with other environmental factors (i.e., viral infections, birth complications, social stressors) acting as the second hit. Feigenson *et al.* (25) later expanded this hypothesis to include inflammation. In the context of inflammation, it has been proposed that MIA could act as an initial trigger, with a second hit (e.g., stress or drugs

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of abuse) exacerbating latent abnormalities (26). Consequently, research is increasingly focusing on the interaction between MIA and cannabis use during adolescence (27–34). It is well established that the endocannabinoid (eCB) system plays a major role in fetal neurodevelopment (35,36); therefore, it is plausible that MIA may disrupt proper development and thereby affect outcomes in adulthood. Recent studies have provided evidence that suggests the involvement of the eCB system in the emergence of neurodevelopmental disorders in preclinical models (37–39). In this review, we discuss the functional implications of the eCB system in MIA models and its involvement in neurodevelopment and the two-hit models of schizophrenia.

eCB SYSTEM

The eCB system consists of a family of lipid molecules, receptors, and enzymes that have been extensively studied in both the central nervous system and the periphery over the last 3 decades. The identification and characterization of key eCBs such as arachidonoylethanolamide (anandamide or AEA) (40) and 2-AG (2-arachidonoylglycerol) (41,42) has been fundamental in elucidating the localization, structure, and function of this system. The discovery and functional expression of cannabinoid receptors such as the CB_1 receptor (CB1R) (43) and CB_2 receptor (CB2R) (44) was a further significant step forward. AEA and 2-AG are synthesized from arachidonic acid–containing membrane phospholipids and feature an ethanolamine or a glycerol moiety, respectively, in their molecule (45–47). Despite their structural similarities, AEA and 2-AG display different receptor affinity for CB1Rs and CB2Rs and distinct biosynthetic and degradation pathways. AEA is primarily synthesized through an NAPE-PLD-dependent pathway, while 2-AG is mainly produced from inositol phospholipids via the combined actions of DAGL and phospholipase C. Although there are multiple potential pathways for the biosynthesis of 2-AG, it is primarily generated by PLCβ acting on membrane phosphatidylinositols, followed by conversion to 2-AG by either of 2 isoforms, DAGLα and DAGLβ. Signaling by eCBs is tightly regulated by metabolic enzymes such as FAAH and MAGL, which predominantly hydrolyze AEA and 2-AG, respectively. In addition to MAGL, 2-AG can be hydrolyzed by two different serine hydrolase ABHD6 or ABHD12. Other enzymes (e.g., NAAA, COX- 2, and several LOX isoenzymes) may also participate in the metabolism of AEA and 2-AG (46). These eCBs are synthesized on demand and travel retrogradely across the synaptic cleft to bind to presynaptic eCB receptors, thereby regulating the release of other neurotransmitters such as glutamate, GABA (gamma-aminobutyric acid), dopamine, serotonin, and acetylcholine (48–50). The on-demand synthesis allows for the modulation of synaptic transmission in various ways (51,52).

ROLE OF THE eCB SYSTEM IN NEURODEVELOPMENT

The eCB system is essential for neurodevelopment from the earliest stages of gestation, affecting the uterus, placenta, and fetal brain (35,36). Beginning around postnatal day 10, the eCB system modulates synaptic transmission (53). The levels of eCBs, their receptors, and associated enzymes fluctuate throughout development (Figure 1). In rodents, CB1R levels peak around postnatal day 30, at the onset of adolescence, particularly in the prefrontal cortex (PFC) and striatum, and begin to decline around postnatal day 70, at the start of adulthood (54,55). However, there is scarce evidence on how eCB signaling shifts during different stages of human development. Previous studies with relatively small sample sizes showed age-dependent changes in CB1R levels in human brains (56,57). Although marked similarities have been observed in the developmental patterns of CB1R expression between humans and rodents, expression within human PFC tissue peaks much earlier (prior to 5 years of age), after which levels gradually decrease until adulthood (58). In rodents, 2-AG levels are high at birth and fluctuate until adolescence, ultimately decreasing overall during this period. In contrast, AEA levels rise gradually until adolescence, peaking between adolescence and adulthood and remain stable in several

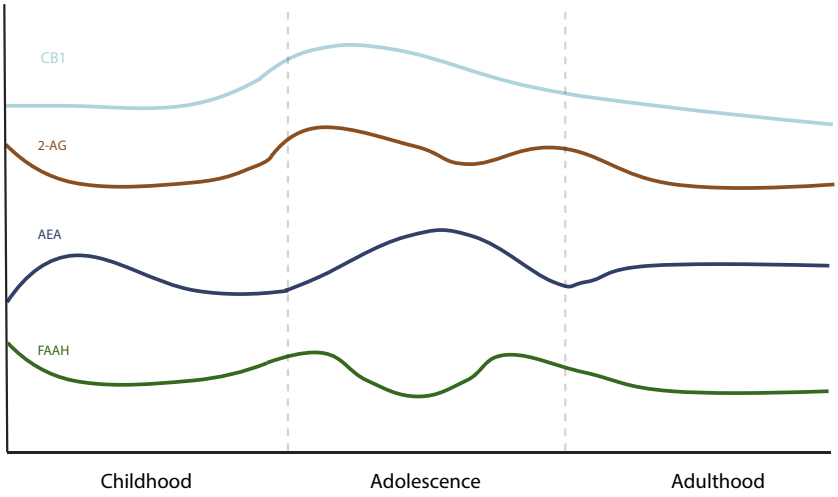

Figure 1. Schematic representation of the corticolimbic endocannabinoid signaling changes dynamically across rodent development. CB_1 levels peak at the onset of adolescence and begin to decline around the beginning of adulthood; 2-AG levels are high at birth and fluctuate until adolescence, ultimately decreasing overall during this period; AEA levels show an opposite trajectory to FAAH. Adapted with permission from (143). 2-AG, 2-arachidonoylglycerol; AEA, arachidonoylethanolamide.

corticolimbic regions such as the hypothalamus, hippocampus, amygdala, and PFC (53). Conversely, FAAH levels show a trend opposite to AEA. Both eCBs and CB1Rs have been observed in white matter areas during prenatal development (59). The pattern of MAGL expression is less well documented, but it appears to decrease from the first year of life in humans, with a more notable decline at the onset of adolescence (58). The changes in the eCB system that take place during pubertal maturation may be linked to interactions between this system and gonadal hormones (54), influencing developmental changes during puberty. In rodents, CB1Rs peak during adolescence and decrease in adulthood, and their higher variations occur in prefrontal and limbic areas (60). These developmental modifications may be influenced by external factors that negatively affect physiological trajectories. Alterations in the eCB system during sensitive periods can produce long-term effects on stress responses later in life. Disrupting the refinement of the maturing system may impair the functionality of the corticolimbic circuit, leading to behavioral changes. It has been shown consistently that exposure to CB1R agonists during adolescence can induce anxiety-related behavior (61,62).

ROLE OF THE eCB SYSTEM IN NEURODEVELOPMENTAL DISORDERS

Increasing evidence indicates that disrupting the eCB system during crucial neurodevelopmental periods can lead to abnormal developmental trajectories and neurodevelopmental disorders (38,63,64). The eCB system plays a crucial role in fetal neurodevelopment, particularly by regulating synaptic plasticity and neuronal cell proliferation and differentiation (35,36). Several meta-analyses and systematic reviews have shown that cannabis use during pregnancy is linked with postnatal neurodevelopmental disorders in children, although these outcomes can be confounded by sociodemographic factors and the use of other substances such as tobacco and alcohol (65–67).

Δ^9-Tetrahydrocannabinol (THC) crosses the placental barrier, making fetal developmental processes and long-term outcomes susceptible to prenatal cannabis exposure. Cognitive impairments have also been observed in animal studies of prenatal cannabis exposure. Both adolescent and adult rodents exposed to THC or a synthetic CB1R agonist in utero exhibit deficits in learning, long-term memory, sensorimotor gating, attention, and spatial working memory compared with controls (68–72). Thus, cannabinoid exposure during pregnancy can alter the maturation of neurotransmitter systems and affect behavior (73). Adult animals exposed to THC perinatally show long-term neurobehavioral disturbances due to disrupted neurotransmitter development patterns (73). These effects are mediated through the activation of CB1Rs, which emerge early in neurodevelopment (74,75) and disrupt neuronal network oscillations and sensory gating in the limbic system, supporting the link between cannabis abuse and increased schizophrenia risk (76,77).

The eCB system's role in the maturation of GABAergic interneuron and dopaminergic cells during prenatal development is critical for the emergence of psychoses in adulthood (38). Disrupting the neurodevelopmental trajectory of the eCB system during the prenatal period may result in neurodevelopmental disorders such as schizophrenia. Moreover, adolescence is crucial as a sensitive period of brain development during which the brain undergoes morphological and functional changes for its maturation. There is ample evidence that the mesocorticolimbic system continues to develop throughout adolescence and achieves final axon density levels during early adulthood (78). However, it is evident that the eCB system is dynamic in the adolescent brain, particularly concerning the development of mesocorticolimbic structures, by regulating the balance between excitatory and inhibitory neurotransmission. During adolescence, the synchronized interaction between limbic and cortical signals is essential for selective information processing. This process shapes the way that inputs from the limbic regions influence the PFC, affecting fear responses and anxiety levels in adolescents (79,80). Moreover, these specific neuroplasticity patterns together with behaviors that characterize adolescence (e.g., increase in novelty-seeking and risk-taking behaviors) may increase vulnerability to consumption of drugs of abuse. In human subjects under 17, cannabis use is correlated with decreased brain volume and a smaller proportion of gray matter, which may be associated with heightened synaptic pruning during adolescent development (81).

TWO-HIT MODELS OF SCHIZOPHRENIA: FOCUS ON MIA AND THC

In the two-hit hypothesis of schizophrenia, it is suggested that MIA may serve as an initial trigger, with a subsequent stressor or drug abuse acting as a second hit that exacerbates latent abnormalities later in life (26). Clinical and epidemiological studies have identified several risk factors for schizophrenia but could not determine the underlying mechanisms. The complexity of this hypothesis is further complicated by factors such as severity, timing, and frequency of stressors. Animal studies are crucial for exploring the mechanisms linked with schizophrenia-related disorders (47,82). Additionally, experimental variables such as stressful stimuli and their severity and timing can be controlled more precisely in rodent models. Several two-hit models in animals have used different combinations of insults at various ages (perinatal, adolescence, or adulthood) and intensities to investigate the etiology of neurodevelopmental disorders (83–86).

The eCB system is crucial for the maturation of the dopamine system. Impairment of the eCB system induced by MIA may lead to abnormal dopamine system development, resulting in schizophrenia-related disorders. An impaired dopamine system is a hallmark of schizophrenia (87,88). Numerous studies have reported alterations in the dopamine system in MIA models, such as changes in firing rate and pattern of dopamine cells, enhanced dopamine release and hyperdopaminergia, and increased sensitivity to psychostimulants (19,89–91) that might persist across generations (92,93). The first hit from poly(I:C) injection activates the TLR pathway, modulating nuclear factor-κB and AP-1 through the MAPK (mitogen-activated protein kinase) cascade. This leads to the synthesis of several proinflammatory cytokines, such as IL-1, IL-6, TNF-α, and IFNs (20). According to the two-hit hypothesis in the context of MIA, a second hit by chronic THC

treatment during adolescence could worsen the dysfunctions that have been observed in adult offspring. However, several studies have not supported this claim (29–33,94). Luchicchi *et al.* (91) found that THC treatment during adolescence attenuated MIA-induced dopamine system disruption. Stollenwerk and Hillard (30) confirmed these observations, with THC treatment during adolescence not potentiating the behavioral effects of MIA in adulthood. Accordingly, Guma *et al.* (31) found no behavioral alterations in MIA THC-exposed rats, although they observed several neuroanatomical alterations. Moreover, THC impaired sociability and social memory, with no interactions between MIA and THC exposure (32). According to these studies, THC-exposed MIA offspring showed significant improvements in sensorimotor gating deficits (33). These findings suggest a need to elucidate the mechanisms that underlie the paradoxical effects of THC. Research should prioritize studying the intricate interaction between the eCB system and neurodevelopmental trajectories in MIA models. MIA may disrupt eCB system development, which in turn may affect the physiological maturation of other systems crucial for the onset of schizophrenia. One plausible hypothesis is that the inflammation caused by MIA interferes with eCB system development, which in turn leads to abnormal dopamine systems in adulthood. The regulation of synaptic transmission by eCBs is altered by an excess of proinflammatory cytokines such as IL-6 and TNF-α, which can lead to neurodevelopmental disorders (95,96). A recent systematic review underscored the importance of the TLR pathway in linking maternal inflammation during pregnancy to neurodevelopmental disorders in human offspring (97). On the other hand, the synthetic cannabinoid WIN55,212–2 acts on TLR3 and TLR4 signaling by inhibiting the proinflammatory response triggered by TLR3 and TLR4, while selectively enhancing TLR3-induced activation of IFN regulatory factor 3 and expression of IFN-β (98). These findings support the notion that poly(I:C) in the MIA model activates the TLR pathway, modulating nuclear factor-κB and AP-1 activation through MAPK cascade and leading to the synthesis of several proinflammatory cytokines, such as IL-1, IL-6, TNFα, and IFNs (20). The actions of WIN55,212–2 on TLR3 and TLR4 signaling may partially explain the inconsistent results that have been obtained in two-hit models (Table 1). Considering the very early-life impact of MIA on the brain as first hit and the later exposure to the second hit during a subsequent period of life (e.g., during adolescence), epigenetic mechanisms may play a crucial role. Several studies have provided evidence for a link between polymorphisms in the *CNR1* gene, which encodes CB1R, and schizophrenia (99). Moreover, alterations of DNA methylation at the promoter of *CNR1* have been described in patients with schizophrenia (100). More recently, research has confirmed the key role of *CNR1* gene regulation in psychosis and proposed that DNA methylation levels at specific sites could serve as potential biomarkers (101). In addition, the expression of the *CNR2* gene has been linked to the comorbidity of schizophrenia and cannabis dependence, while polymorphisms in the *FAAH* gene have been associated with cannabis dependence in a Spanish population (102).

These genetic and epigenetic changes are particularly relevant in the context of schizophrenia, with adolescent cannabis use exerting specific effects on these abnormalities (103).

2-AG SIGNALING IN eCB-MEDIATED SYNAPTIC PLASTICITY AND NEUROINFLAMMATION

Several animal models of psychiatric diseases that utilize different prenatal insults have demonstrated altered eCB-mediated synaptic plasticity, together with enhanced 2-AG signaling, in mesolimbic structures. This suggests a common disrupted pathway that leads to neurodevelopmental impairment (39,70,104–107). An imbalance in 2-AG levels leads to disrupted eCB-mediated synaptic plasticity in mesolimbic structures, ultimately causing abnormalities in the dopamine system during adulthood that resembles a schizophrenia-like endophenotype (39).

Similarly, Guo *et al.* (108) observed region-specific increases in 2-AG signaling, indicating that prenatal immune activation can cause long-term alterations in eCB-mediated plasticity, particularly affecting inhibitory synaptic transmission in the hippocampus. Notably, 2-AG exerts anti-inflammatory effects through CB1R/CB2R and PPAR (peroxisome proliferator-activated receptor) (109). Given that 2-AG acts as a reservoir for arachidonic acid during inflammatory conditions (109), it is hypothesized that increased 2-AG levels may be an adaptive response to counteract elevated proinflammatory cytokines caused by MIA (Figure 2). Supporting this idea, 2-AG has been shown to inhibit proinflammatory cytokine production in lipopolysaccharide-activated rat microglial cells, murine splenocytes, and peritoneal macrophages, as well as having antiproliferative effects on lymphocytes (110–113). The neuroprotective effects of 2-AG are largely linked to its ability to suppress neuroinflammation (114). 2-AG also protects the blood-brain barrier after closed head injury and inhibits messenger RNA expression of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) (115).

Enhancing 2-AG signaling by inhibiting its degradation has emerged as a promising strategy, with MAGL, the main enzyme that degrades 2-AG, becoming an attractive drug target. The neuroprotective effects of MAGL inhibitors are likely due to increased 2-AG signaling and decreased eicosanoid levels. This suggests that controlling 2-AG degradation through MAGL could be crucial for reducing neuroinflammation (116).

Moreover, 2-AG levels may be regulated via a COX-2–dependent mechanism, potentially alleviating anxiety- and depressive-like states in rodents (117,118). Elevated 2-AG levels have been reported consistently in antipsychotic-free patients in the PFC and hippocampus (119). These higher levels may represent an adaptive response to mitigate glutamatergic hyperactivity observed in the brain of individuals with schizophrenia. In this context, it has been reported that 2-AG selectively depresses the increase in firing and bursting activity of dopamine neurons induced by PFC stimulation (120).

Persistent excessive release of 2-AG by platelets has also been suggested as a possible factor that contributes to cognitive impairments related to the negative symptoms of schizophrenia (121). Moreover, increased 2-AG levels have been documented in postmortem human PFC in individuals with schizophrenia (122). Accordingly, a recent study showed a correlation between depression severity and elevated 2-AG levels in the serum of patients with depression, reinforcing the link between inflammation and depressive symptoms (123).

Table 1. Two-Hit Models Based on MIA and Cannabinoid Treatments

Reference	Dams	First Hit	Second Hit	Results
(30)	Sprague Dawley rats	Poly(I:C) (4 mg/kg i.v.) GD 15	THC injections at PND 45–55 in increasing doses (2.5–10 mg/kg), twice per day Male offspring	THC treatment rescued the effects of MIA on VTA dopamine neurons.
(31)	C57BL/6J mice	Poly(I:C) (5 mg/kg i.p.) GD 12	THC was administered orally through voluntary consumption of a cereal piece containing THC (30 μL of 2 mg/mL in ethanol) at PND 35–39 Male and female offspring	THC treatment during adolescence failed to potentiate the behavioral effects of MIA in adulthood.
(32)	C57BL/6J mice	Poly(I:C) (5 mg/kg i.p.) GD 9	THC injections at PND 28–45 (5 mg/kg i.p.) daily Male and female offspring	Lack of behavioral alterations in MIA THC-exposed rats Neuroanatomical alterations due to the combination of MIA and THC exposure
(33)	Sprague Dawley rats	LPS (0111:B4 50 μg/kg) GD 15 and GD 16	THC injections at PND 28–44 (3 mg/kg i.p.) every other day Male and female offspring	THC impaired sociability and social memory, but there were no interactions between MIA and THC exposure.
(34)	Wistar rats	PolyI:C (4 mg/kg i.v.) GD 15	THC injections at PND 28–38 (10 mg/kg i.p.) daily Male offspring	THC-exposed MIA offspring showed significant improvements in sensorimotor gating deficits.
(35)	Sprague Dawley rats	MAM (22 mg/kg i.p.) GD 17	THC injections from PND 29–39, twice/day with increasing doses (2.5–5–10 mg/kg i.p.) Male offspring	THC reversed cognitive deficits in MAM offspring by modulating *Drd2* and *Drd3* gene expression.
(28)	Sprague Dawley rats	MAM (20 mg/kg, i.p.) GD 17	WIN injections from PND 40–65 (1.2 mg/kg i.p.) Male offspring	WIN did not exacerbate the behavioral and electrophysiological changes in MAM offspring but attenuated the increased locomotor response to amphetamine.
(29)	Sprague Dawley rats	MAM (25 mg/kg i.p.) GD 17	WIN injections from PND 35–45 (0.2 mg/kg i.p.) every other day Male and female offspring	WIN increased the proportion of second-generation MAM rats that developed schizophrenia-like deficits.
(94)	Wistar rats	Poly(I:C) (5 mg/kg i.v.) GD 19	HU-210 injections from PND 35–48 (0.1 mg/kg for males and 0.075 mg/kg for females, i.p.) Male and female offspring	Reduced amplitude of human-like mismatch responses in male rats of both single-hit and two-hit groups

This table summarizes studies that have investigated the two-hit hypothesis in the context of MIA and cannabinoid exposure. Each study listed examined the effects of a primary hit [MIA induced by agents such as poly(I:C) or LPS] and a secondary hit (cannabinoid treatments such as THC or synthetic cannabinoids) on different animal models. The table includes details on the animal models used, the nature and timing of the first and second hits, and the resultant effects observed in terms of neuroanatomical, behavioral, and neurochemical outcomes. These studies collectively aimed to elucidate the complex interactions between MIA, the endocannabinoid system, and neurodevelopmental disorders.

GD, gestational day; i.p., intraperitoneal; i.v., intravenous; LPS, lipopolysaccharide; MAM, methylazoxymethanol acetate; MIA, maternal immune activation; PND, postnatal day; THC, Δ^9-tetrahydrocannabinol; VTA, ventral tegmental area.

These findings indicate altered eCB metabolism in this psychiatric disorder. However, using 2-AG as a biomarker to predict neurodevelopmental disorders presents challenges because the quantification of 2-AG in blood samples is insufficiently stable (124). Therefore, it is essential to establish robust preanalytical protocols to ensure reliable assessment across samples. Technical refinements, such as using aprotic solvents and GlucoEXACT blood collection tubes (Sarstedt AG), can minimize the isomerization of 2-AG to 1-AG (125).

To conclude, 2-AG is highly relevant due to its implication in various physiological processes and its potential association with pathological conditions. It may represent an intriguing therapeutic target for schizophrenia and other neuropsychiatric disorders (124).

ROLE OF THE eCB SYSTEM AS A THERAPEUTIC TARGET FOR SCHIZOPHRENIA: FOCUS ON CLINICAL STUDIES

Progress in our knowledge of the eCB system has driven pharmaceutical research to investigate whether manipulating this system could offer therapeutic benefits (126). Full coverage of human and clinical studies conducted on patients with schizophrenia and potential therapeutics of cannabinoids is beyond the scope of this review [for excellent reviews on clinical trials/human studies, see (127–130)]. The most promising preliminary findings are related to the use of cannabidiol (CBD) in psychotic symptoms and anxiety. CBD is one of the 2 main phytocannabinoids present in the *Cannabis sativa* plant,

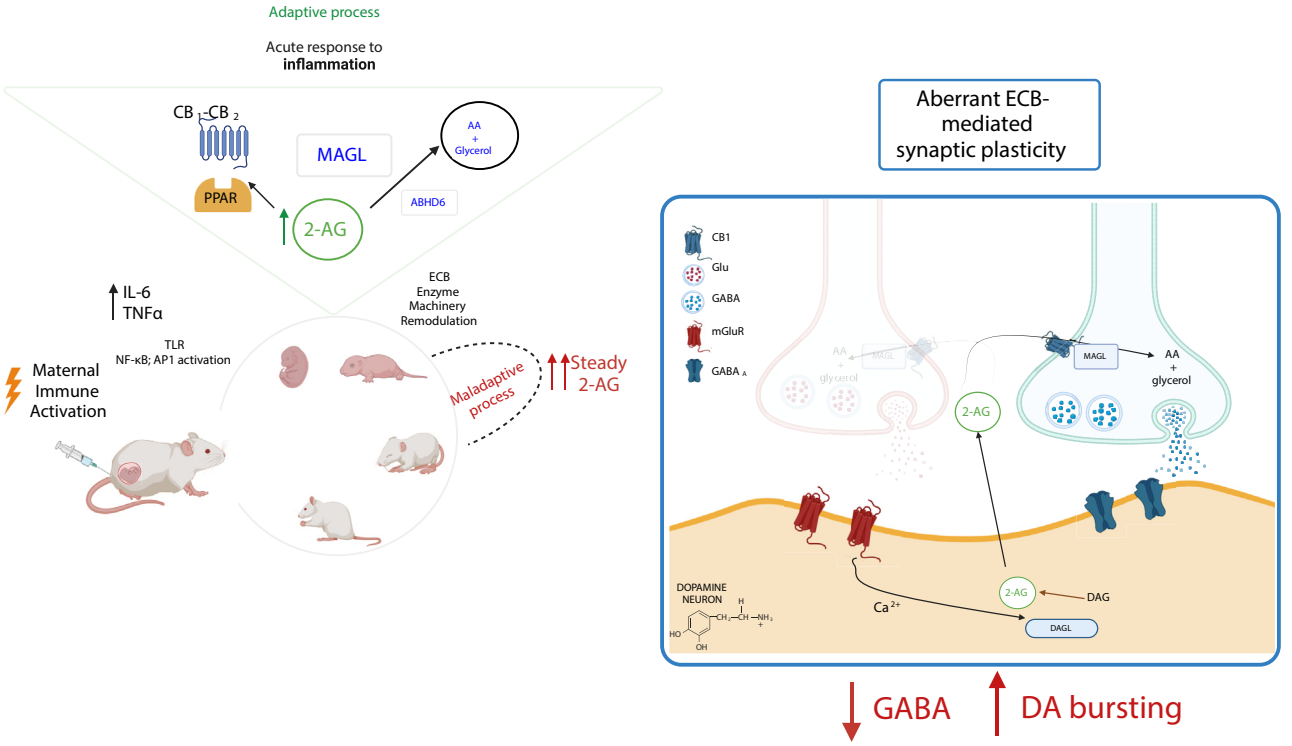

Figure 2. This figure illustrates the proposed mechanisms by which maternal immune activation impacts the ECB system, specifically 2-AG signaling, contributing to neurodevelopmental disorders. Maternal immune activation, through the activation of TLRs and subsequent NF-κB and AP1 pathways, induces the production of proinflammatory cytokines such as IL-6 and TNF-α. Key components of the ECB system, including 2-AG, are depicted within an adaptive and maladaptive process framework. In the adaptive process, an enhanced 2-AG signaling involves binding to cannabinoid receptors (CB_1 and CB_2) and PPARs, with the involvement of metabolic enzymes such as MAGL and ABHD6 for 2-AG breakdown into AA and glycerol. The maladaptive process highlights the disruption of ECB-mediated synaptic plasticity due to steady elevated levels of 2-AG in response to maternal immune activation–induced inflammation. This disruption affects various neurotransmitter systems, including Glu and GABA, and leads to aberrant synaptic plasticity, particularly in dopaminergic neurons, which is implicated in the pathophysiology of schizophrenia. 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; ECB, endocannabinoid; $GABA_A$, gamma-aminobutyric acid A; Glu, glutamate; IL, interleukin; mGluR, metabotropic glutamate receptor; NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator-activated receptor; TLR, toll-like receptor; TNF-α, tumor necrosis factor α.

and it does not have psychotropic effects. Although it does not bind to CB1R or CB2R, it may affect the extended endocannabinoidome by interacting with multiple targets (131). It has been approved by the Food and Drug Administration and the European Medicines Agency for the treatment of seizures that occur in 2 rare diseases, Dravet syndrome and Lennox-Gastaut (131). The first antipsychotic effects of CBD were documented in a patient with schizophrenia who was treated with CBD, resulting in a reduction in psychotic symptoms (132). The first double-blind, randomized clinical trial of CBD versus amisulpride showed that CBD or amisulpride was accompanied by an increase in serum AEA levels, which was significantly associated with clinical improvement (133). In a recent randomized clinical trial with CBD, patients showed significantly different neural activation compared with healthy control individuals on a verbal recall task. However, CBD improved activation patterns but failed to reach the results of the healthy control individuals (134). An ongoing phase II randomized, double-blind clinical trial (NCT04105231) aims to evaluate the efficacy of CBD versus risperidone, a first-choice second-generation antipsychotic, in patients with early psychosis and comorbid cannabis use. Moreover, a double-blind, randomized clinical trial conducted in patients with acute paranoid schizophrenia receiving either CBD or amisulpride showed that both improved cognitive functioning with comparable efficacy in patients with schizophrenia via an AEA-independent mechanism (135). In the context of modulators that orchestrate the eCB enzyme machinery, FAAH is the only enzyme that has been specifically explored (136). It has been observed that brain FAAH in psychosis may represent a biomarker of disease state and a novel target for interventions to treat psychotic symptoms (137). On the other hand, selective inhibitors for MAGL, which metabolizes 2-AG, or compounds that inhibit both enzymes have not yet been studied in humans.

LIMITATIONS

In the case of the two-hit models of schizophrenia, the multitude of factors associated with the emergence of the disorder (e.g., the severity of stress, timing of exposure, number of stressors) adds significant complexity to this framework. Here, we acknowledge a few noteworthy limitations. First, findings derived from animal models may not fully capture the intricacies of human physiology and neurodevelopmental complexities, limiting translational applicability. Additionally, our

review focuses on specific eCB pathways, potentially overlooking other critical molecular mechanisms involved in schizophrenia. Moreover, methodological inconsistencies might also have contributed to the contradictory results observed across studies. For example, Table 1 illustrates the lack of standardization in MIA two-hit models (e.g., first and second hit, THC or synthetic cannabinoids, doses, neurodevelopmental window, gestational timing, and animal strain). On the other hand, the lack of standardization represents an opportunity for additional exploration. Nevertheless, it remains crucial to report detailed methodologies to enhance rigor and reproducibility across laboratories and species (138). To thoroughly elucidate the molecular mechanisms and long-term behavioral outcomes in human populations affected by MIA and THC/synthetic cannabinoid exposure, more comprehensive and longitudinal studies are needed that span both preclinical and clinical research.

SUMMARY AND CONCLUSIONS

Reciprocal regulation between the eCB system and neurodevelopment has been documented. As reviewed here, several two-hit models of schizophrenia failed to exacerbate MIA-related abnormalities. However, the precise mechanisms by which eCB system activation during adolescence attenuates the effects of MIA remain to be elucidated. Dynamic variables such as CB1R agonists suppressing TLR3 and TLR4 signaling by inhibiting the proinflammatory pathway (98) have been reviewed. Treatments aimed at reducing inflammation during critical neurodevelopmental periods prevent behavioral, electrophysiological, and neurochemical deficits in MIA models of schizophrenia (139–141). Altered eCB signaling during adolescence in MIA offspring may also be a plausible explanation (39). Further research could explore the molecular effects of compounds that target the eCB system, such as CBD and FAAH inhibitors, in relation to psychosis. This would help improve our understanding of their potential impact on schizophrenia. Furthermore, given the impact of cannabis on schizophrenia, driving earlier psychosis onset in users (142), future studies should test higher and standardized doses of THC/synthetic cannabinoids to elucidate the interaction between MIA and cannabinoid treatments during sensitive developmental periods.

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From the Department of Biomedical Sciences, University of Cagliari, Monserrato, Italy (MS, MP); Unit of Clinical Pharmacology, University Hospital, Cagliari, Italy (MS, MP); and Neuroscience Institute, National Research Council of Italy, Cagliari, Italy (MP).

Address correspondence to Marco Pistis, M.D., at mpistis@unica.it.

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