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Review

The endocannabinoid system in critical neurodevelopmental periods: sex differences and neuropsychiatric implications

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Abstract

This review focuses on the endocannabinoid system as a crucial player during critical periods of brain development, and how its disturbance either by early life stressful events or cannabis consumption may lead to important neuropsychiatric signs and symptoms. First we discuss the advantages and limitations of animal models within the framework of neuropsychiatric research and the crucial role of genetic and environmental factors for the establishment of vulnerable phenotypes. We are becoming aware of important sex differences that have emerged in relation to the psychobiology of cannabinoids. We will discuss sexual dimorphisms observed within the endogenous cannabinoid system, as well as those observed with exogenously administered cannabinoids. We start with how the expression of cannabinoid CB_1 receptors is regulated throughout development. Then, we discuss recent results showing how an experimental model of early maternal deprivation, which induces long-term neuropsychiatric symptoms, interacts in a sex-dependent manner with the brain endocannabinoid system during development. This is followed by a discussion of differential vulnerability to the pathological sequelae stemming from cannabinoid exposure during adolescence. Next we talk about sex differences in the interactions between cannabinoids and other drugs of abuse. Finally, we discuss the potential implications that organizational and activational actions of gonadal steroids may have in establishing and maintaining sex dependence in the neurobiological actions of cannabinoids and their interaction with stress.

Keywords

Adolescence, animal models, cannabinoid receptors, early life stress, neuropsychiatric disorders, sexual dimorphisms

Preliminary considerations

Psychiatric disorders are a major burden for healthcare systems. Their high prevalence and social impact, especially for caregivers, demand a major effort for their prevention and treatment. Understanding the nature and origin of major psychiatric disorders is one of the most challenging enterprises of modern biomedical research because they are not simple results of genetic defects, nor inevitable outcomes of life events. The interaction between genetics and environment during crucial phases of development, including early postnatal life and adolescence, seems to be the starting point of endophenotypes of vulnerability to mental health problems. As shown in Figure 1(A), the impact of specific external factors upon a genetically determined programme of development can set the stage for the triggering of a psychiatric disorder by external factors in adulthood. The present review focuses on the role of the endogenous cannabinoid system (ECS) as a target for both genetic determinants of neural development and epigenetic alterations of brain maturation, especially during adolescence.

Why animal models are useful in neuropsychiatry

One obvious limitation in developing animal models for psychiatric diseases is that, due to the nature and complexity of human symptoms, it is impossible to reproduce a disease in its entirety. However, several experimental models have been developed in an attempt to mimic specific signs of various neuropsychiatric disorders. These procedures allow experimental controls that are not possible in human studies, and provide a valuable approach for the investigation of neurobiological substrates.

The present review dedicates special attention to the impact of early postnatal life events, focusing mostly on animal studies that investigate the importance of the adolescence phase. Adolescent behaviours are shared across species; for example, adolescent rodents exhibit a particular

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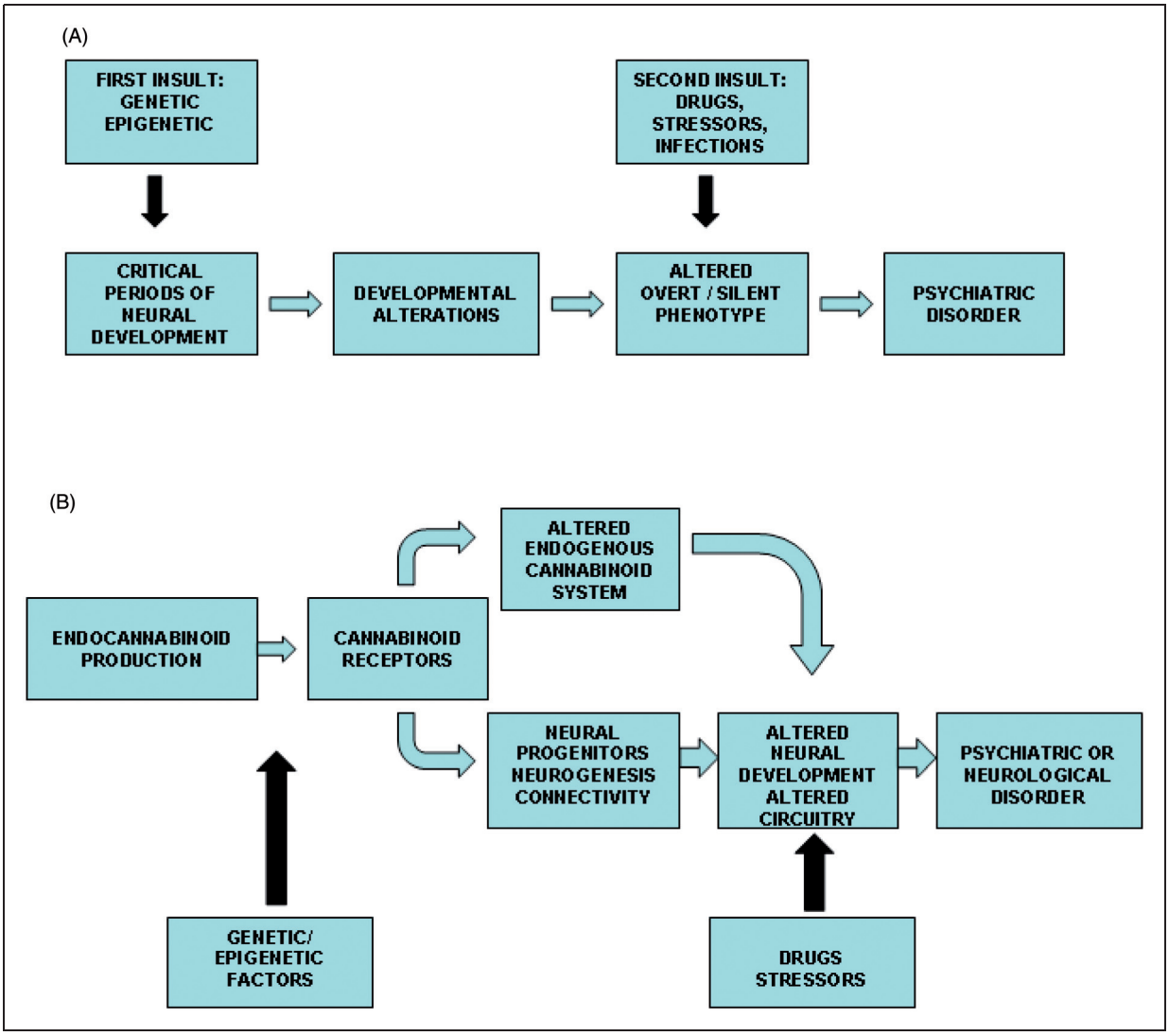

Figure 1. (A) Neural development may be disrupted by the action, during critical periods of life, of both endogenous (i.e. mutations or alterations in the genetic contribution to normal development) or exogenous factors (i.e. epigenetic consequences of early life events, drugs, hormones/endocrine disruptors, infections, etc). This disruption may lead to a new phenotype that may be either overtly expressed or silent. The impact of a second insult in these altered phenotypes (i.e. exposure to stressors, drugs of abuse, etc) may lead in adult life to the onset of a psychiatric disorder. (B) The ECS has been described as being one of the targets for the model described above. Either genetic alterations on endocannabinoid signalling (i.e. mutation on the endocannabinoid degradatory enzyme ABH12 in humans) or the impact of epigenetic factors (i.e. maternal deprivation, mild stress, exposure to drugs) may lead to overt/silent vulnerable phenotypes. Because of the well-known role of the ECS on neural development and plasticity (modulation of neurogenesis, fate of neural precursors, survival, neuritogenesis and connectivity), the impact of genetic/epigenetic factors during critical period may lead to altered circuitry in both non-cannabinoid (i.e. dopaminergic) and/or cannabinoid signalling processes in adulthood. This new vulnerable phenotype has been proposed to contribute to the appearance of psychiatric disease when a concurrence factor (a new stressor, abuse of drugs) occurs during adult life.

behavioural repertoire that resembles human adolescent behaviour, including high levels of exploration, novelty and sensation seeking, impulsivity and an increased sensitivity to incentives. These behaviours have been suggested to help adolescents develop the social skills needed when they become independent from their family or become adults in their group. High levels of novelty/sensation-seeking behaviours appear to be strong predictors of drugs use among adolescents. In addition, while the brain's emotion-related areas and connections are still maturing, adolescents are more vulnerable to psychological disorders (for a review see Adriani and Laviola, 2004; Crews et al., 2007; Laviola et al., 2003; Spear, 2000). Studying the adolescent phase of animals as a model of human adolescence is thus useful for investigating the risk for addictive and other early onset neuropsychiatric disorders. The increasing

risk of developing these disorders that emerge during adolescence has encouraged the investigation of their neurobiological basis, and this particular topic might serve to highlight aberrations in the key developmental domains of cognition, affect and motivational behaviour. While no animal model can represent the full phenotypic spectrum of a psychiatric disorder, such as schizophrenia or depression, specific phenotypic components of disorders can be used to design adequate animal models that are useful to unravel disease mechanisms and that may allow testing novel therapeutic interventions (Adriani and Laviola, 2004; Giedd et al., 2008).

Genetic and epigenetic factors: the key for silent phenotypes

The main psychiatric disorders, and schizophrenia in particular, are thought to be disorders of brain development. As the brain approaches its adult anatomical and physiological state in adolescence, both genetic and environmental factors may initiate a pathological process that leads to the emergence of the disease (for a review see Fatemi and Folsom, 2009). Any animal model designed to test or investigate the neurodevelopmental hypothesis of mental disorders must consistently consider the impact of genetic and environmental factors. Furthermore, this consideration has to address a very important aspect: the incidence of major mental disorders (i.e. depression, psychosis, bipolar disorders) peaks soon after the end of brain maturation, after adolescence. As happens in humans, the animal model has to provide a 'silent phenotype' that may be revealed in adulthood by challenging the animal with external factors, including environmental manipulations (i.e. housing, feeding, temperature), stressors, drug administration, and so on. This model may be addressed either in genetically modified animals or in environmentally reprogrammed ones, that is, animal models of exposure to controlled environmental factors that may shift phenotypic expression. Current methods allow control of the impact of external factors from preimplantation stages to adolescence. For instance, it has been demonstrated that the *in vitro* culture of embryos (i.e. within *in vitro* fertilization technologies) may lead to different phenotypes depending on the presence of serum in the culture media (Fernández-González et al., 2004). Prenatal stress (maternal immobilization) or early postnatal stress (maternal deprivation) may produce long-term changes in the brain of rodents that resemble phenotypic alterations found in humans. A major effort is now focussed on identifying the key elements of such genetic–epigenetic alterations that accumulate during development of human pathologies. Major candidates for such silent phenotypic alterations include signalling systems that target either transcriptional regulation of genes relevant for development, neural proliferation or cell fate and survival. Additional targets include genes controlling neural plasticity and neurite connectivity. One such signalling system is the ECS, which has been described as being involved in main stages of neural development and activity.

Developmental aspects of the endocannabinoid system

The CB_1 cannabinoid receptor is a key component of the ECS, which consists of endogenous ligands called endocannabinoids, typically anandamide (AEA) and 2-arachidonylglycerol (2-AG), which act upon activation of cannabinoid receptors (CB_1 and CB_2 receptors) as well as synthesizing and degrading enzymes and potential endocannabinoid membrane transporters. CB_1 receptor is the predominant cannabinoid receptor within the central nervous system, and is highly expressed in brain regions involved in emotional processing, motivation, motor activation and cognitive function (Mackie, 2005). In addition to cannabinoid receptors, which are the most-studied elements of the ECS, there are many other genes involved in endocannabinoid production and degradation whose role is only beginning to be understood. Some of these genes were specifically described as genes regulating axonal growth and guidance, such as diacyl glycerol lipase alpha, one of the major enzymes in 2-AG production (Bisogno et al., 2003; Goncalves et al., 2008). Genetic deletion of monoacyl glycerol lipase or ABHD6, two of the main enzymes degrading endocannabinoids, causes endocannabinoid overload, resulting in altered developmental phenotypes (for a review see Blankman et al., 2007; Lichtman et al., 2010; Marrs et al., 2010). Interestingly, the first inherited defect of endocannabinoid signalling described in humans has been reported recently and affects one of the enzymes, ABHD12 hydrolase, which degrades 2-AG. The defect produces the PHARC syndrome characterized by polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (Fiskerstrand et al., 2010), although further studies are needed to clarify the role of the endocannabinoid contribution to this syndrome.

Among the multiple functions of the ECS there is evidence for its role in neural development (Bermudez-Silva et al., 2010; Cota, 2008; Moreira and Lutz, 2008; Viveros et al., 2005a, 2005b, 2007; Wotjak, 2005), as well as its participation in the specification of connectivity patterns (Galve-Roperh et al., 2008; Mulder et al., 2008; Wu et al., 2010). Both CB_1 receptors and endocannabinoid ligands can be detected in the rat (Belue et al., 1995; Rodriguez de Fonseca et al., 1993) and human (Mato et al., 2003) brain during early developmental periods (Belue et al., 1995; Mato et al., 2003; Rodriguez de Fonseca et al., 1993; Viveros et al., 2005a). In animal models, AEA content has been observed to gradually increase during early postnatal stages, reaching its maximum in the adolescent brain (Harkany et al., 2007). Similarly, in rat brain CB_1 receptors exhibit a mainly postnatal pattern of development, reaching maximal densities during adolescence which later drop to adult expression levels, as detected in the dorsal striatum (Belue et al., 1995; Rodriguez de Fonseca et al., 1993).

During the perinatal period, a common atypical pattern of CB_1 receptor expression has been found both in rodents and humans; high densities of CB_1 receptors have been observed in fibre-enriched areas that are practically devoid of them in the adult brain. This transient pattern of CB_1 receptor localization in white matter areas during the prenatal stages suggests a specific role of the ECS in neural development, which may

be important for guidance processes that result in the establishment of cortical–subcortical connections (Belue et al., 1995; Mato et al., 2003; Rodriguez de Fonseca et al., 1993). At early developmental stages, the ECS seems both to influence the appearance of key cellular signals and to modify the expression of genes that are relevant for neural development. These ECS-mediated actions also involve axonal growth, fasciculation and the establishment of correct neuronal connectivity (Fernandez-Ruiz et al., 2004; Harkany et al., 2007; Watson et al., 2008). In particular, during early phases of neuronal development, endocannabinoid signalling is integral for an array of processes including the proliferation and differentiation of progenitor cells, neuronal migration, axonal guidance, fasciculation, positioning of cortical interneurons, neurite outgrowth and morphogenesis (Harkany et al., 2007, 2008a, 2008b). The importance of the ECS during early developmental periods is further supported by the aberrations that occur following disruption of normal endocannabinoid signalling during ontogenetic phases. For example, pharmacological blockade of the CB_1 receptor in mid-to-late gestational periods impaired progenitor proliferation in the subventricular zone, disrupted axonal pathfinding and resulted in cortical delamination (Mulder et al., 2008), whereas, in utero exposure to Δ-9-tetrahydrocannabinol (THC) hampered appropriate interneuron positioning during corticogenesis and resulted in an increase in the density of CCK-positive interneurons in the hippocampus (Berghuis et al., 2005).

In humans, expression patterns of the CB_1 receptor have been found to increase dramatically from infancy to young adulthood in regions such as the frontal cortex, striatum and hippocampus (Mato et al., 2003). Rodent studies have provided further time- and region-specific data. We showed that the ontogeny of the receptors in rat striatum, limbic forebrain and ventral mesencephalon was relatively similar, exhibiting a progressive increase that peaks on days 30 or 40 and then subsequently decreases to adult values (Rodriguez de Fonseca et al., 1993). In the female hypothalamus, AEA levels are seen to peak at the onset of puberty and then decline into adulthood (Wenger et al., 2002). More recent studies have revealed clear developmental fluctuations throughout adolescence in endocannabinoid levels in the nucleus accumbens (NAcc) and prefrontal cortex (PFC), brain regions involved in reward, motivation, and cognition. The most profound alteration was the continuous increase in PFC AEA levels throughout the adolescent period; concentrations were almost three times higher in late than early adolescence. However, 2-AG concentrations were lower in the PFC in the later phases than in the beginning of the adolescent period, a finding paralleled in the NAc. In addition, CB_1 receptors were found to vary in the PFC and NAc core during the different phases of adolescence, although the alterations were less marked than for endocannabinoid levels. These findings emphasize dynamic alterations in endocannabinoid function in mesocorticolimbic regions of the adolescent brain that are relevant to reward and, to an even greater extent, cognition and emotional learning, and underscore the specific association of the ECS with neurodevelopment, not only for the perinatal period but also during adolescence (Ellgren et al., 2008). Whereas most data available in the literature refer to expression of protein or mRNA for brain CB_1 receptors, it would be extremely interesting to examine the developmental changes of CB_1 receptor functional activity throughout these critical developmental periods. It is plausible that the neurodevelopmental and morphogenic roles of endocannabinoids somehow continue in adolescence, and it seems quite likely that disruption of normative endocannabinoid signalling during this time period has long-term functional consequences on adult brain function and behaviour. The implication of the ECS in brain developmental processes may explain the negative effects of cannabinoid consumption in adolescence on emotional and cognitive function, as well as cognitive deficits observed in children born to women who used marijuana during pregnancy (Mereu et al., 2003).

In our developmental study quoted above (Rodriguez de Fonseca et al., 1993), we found subtle sexual dimorphisms in the rat striatum and ventral mesencephalon but not the limbic forebrain, and we did not find significant differences in the expression of hippocampal CB_1 receptors among neonatal rats (Suarez et al., 2009). At adolescence (postnatal day (PND) 43), subtle differences in the expression of hippocampal CB_1 receptors were found, with female rats showing lower cannabinoid CB_1 receptor density when compared with males (Marco et al., 2007b). In contrast, clear sex differences in CB_1 receptors are evidenced in adult rats that have been described for both the expression and the functionality of hippocampal CB_1 receptor. Male rats show higher levels of hippocampal CB_1 receptor expression than females (Reich et al., 2009), whereas female rats exhibit a pattern of higher CB_1 receptor-mediated G protein activation in hippocampal formation when compared with male animals (Mateos et al., 2010). Thus, it seems likely that sexual differences in CB_1 receptor expression (at least in certain regions such as the hippocampus) are established beyond PND 40. Interestingly, however, differential effects of diverse kinds of stress on hippocampal CB_1 receptor expression of male and female rats have been found in both adult (Reich et al., 2009) and 13-day-old neonatal animals (Suarez et al., 2009), suggesting a role for organizational sex steroids during the perinatal period.

Interaction of early life stress with the endocannabinoid system

Impact of early stress

Evidence indicates that traumatic experiences during early developmental periods might be associated with psychopathology (such as depression or schizophrenia) and altered neuroendocrine function later in life (Levine, 2005; Moffett et al., 2007; Tyrka et al., 2008), and several experimental models have been developed in an attempt to mimic diverse types of early life stress. One of the multiple mechanisms by which these traumatic episodes affect brain development is the activation of stress responses including glucocorticoid receptors. Hormonal receptors are one of the most important targets for epigenetic alterations of the developmental programme. A main group of hormonal receptors are transcription factors regulating gene expression. There is much evidence that such receptors are involved in neurogenesis, neural fate, differentiation and survival. Loss or inappropriate secretion of these hormones often results in profound

alterations in brain circuitry, affecting motor development, sexual differentiation of the brain or cortical patterning (see McCarthy, 2008 for a review on oestradiol actions on neurodevelopment). Glucocorticoids, thyroid hormones and sex steroids are among the hormones found to regulate brain development, and all clearly affect the ECS (Asúa et al., 2008; Mailleux and Vanderhaeghen, 1993; Rodriguez de Fonseca et al., 1994). For instance, congenital hypothyroidism is associated with impaired expression of the cannabinoid CB_1 receptor in the striatum, resulting in hyperactivity. Administration of thyroid hormone to hypothyroid pups returns both spontaneous activity and CB_1 expression to normal levels (Asúa et al., 2008). As described in multiple observations, early life stress affects the ECS leading to alterations in emotional processing, stress responses, dopaminergic function, and so on (del Arco et al., 2000; Moreno et al., 2005; Rubio et al., 1995). We are beginning to unveil how stress impacts on the ECS and how its disruption affects permanently brain functions (Figure 1(B)). This example also serves to highlight the difficulties in finding appropriate controls for the developmental hypothesis, since minimal manipulations may severely affect certain brain functions, acting as confounding variables.

Maternal deprivation as a suitable animal model

One of the best-studied models of early life stress is maternal deprivation (MD). Notably, adult rats submitted to a 24-h episode of MD at PND 9 showed behavioural abnormalities that resembled psychosis-like symptoms, including disturbances in pre-pulse inhibition (PPI), latent inhibition and auditory sensory gating, and startle habituation. Regarding possible underlying neurochemical correlates, several neurotransmitters systems involved in mental disorders have been analysed. They include peptides such as neuropeptide Y (NPY), involved in affective disorders, or glutamate transmission through the N-methyl-D-aspartate receptor, hypothesized to be involved in positive and negative symptoms of schizophrenia (Labrie and Roder, 2010).

Adult MD animals showed reduced levels of NPY in the occipital cortex and hippocampal formation, and a significant reduction in hippocampal levels of calcitonin-gene-related peptide, polysialylated neural cell adhesion molecule and brain-derived neurotrophic factor, and a significant decrease in the mRNA levels of glutamate N-methyl-D-aspartate receptor subunits NR-2A and NR-2B. These changes are suggestive of a loss of synaptic plasticity and hypofunctionality of the glutamatergic system, as recently postulated for schizophrenia (for a review see Ellenbroek and Riva, 2003; Ellenbroek et al., 2004). In addition we have shown that, at adolescence, MD rats showed depressive-like behaviour, a trend towards increased impulsivity (Marco et al., 2007a) and altered behavioural, immune and endocrine responses to cannabinoid agonists such as WIN 55,212-2 (Llorente et al., 2007; Marco and Viveros, 2009). One of the most prevalent hypotheses for the pathogenesis of schizophrenia states that the disease is a neurodevelopmental disorder associated with early brain developmental abnormalities (Lewis and Levitt, 2002; Marek and Merchant, 2005; Weinberger, 1987). From this perspective, we expected that behavioural deficits observed in mature MD animals might be related at least partially to altered neural development, possibly triggered by stress-induced increases in glucocorticoid levels. Some of these effects resemble those observed after pharmacological manipulation of glucocorticoid tone during late gestation and early lactation periods, indicating that glucocorticoid-derived actions may be mediating part of the response to MD. In fact, MD acutely leads to high levels of corticosterone (Suchecki et al., 1993) that remain elevated at PND 13 in MD male and female rats (Llorente et al., 2008; Viveros et al., 2009). In accordance with our hypothesis, we described sex-dependent alterations in developing hippocampal and cerebellar neurons and glial cells in MD neonatal rats, with males being more markedly affected. Changes included alterations of endocannabinoid levels, number of astrocytes and corticosterone levels (Llorente et al., 2008, 2009; López-Gallardo et al., 2008). These alterations appear to support our claim that MD neonatal stress may be a potential model to analyse neuropsychiatric symptoms with a basis in neurodevelopment.

Several lines of evidence support an association between an altered ECS and the pathogenesis of schizophrenia. For example, increases in CB_1 cannabinoid receptor expression have been found in the prefrontal cortex (Dean et al., 2001) and cingulate cortex (Zavitsanou et al., 2004) of schizophrenic patients. Also, elevated levels of the endocannabinoid AEA have been detected in the cerebrospinal fluid of schizophrenics (Giuffrida et al., 2004; Leweke et al., 1999, 2007). Moreover, frequent cannabis use significantly increases the risk for psychotic symptoms and schizophrenia (for a review see Di Forti et al., 2007; Leweke and Koethe, 2008). In addition, as indicated above, the ECS appears to play a major role in brain development. Based on the previously reported psychosis-like symptoms in adult MD animals and on our findings of cellular changes in relevant brain regions such as the hippocampus, we expected that MD might also induce alterations of the ECS. Our results confirmed that neonatal MD animals showed an increased level of the endocannabinoid 2-AG and a decrease in hippocampal CB_1 immunoreactivity. It is important to stress that findings in both human and animal models point to the participation of the ECS in the pathogenesis of schizophrenia (or behavioural phenotypes that serve as model of this disease). The nature of the changes (location, intensity, direction, etc) and the impact on cognitive and executive functions need to be addressed in depth, taking into account the important species differences that exist between rodents and humans.

These alterations were more marked in male animals (Suarez et al., 2009). Concordant with the increased 2-AG levels, we have found more recently that MD also induced a significant decrease in hippocampal monoacylglycerol lipase, the enzyme involved in the degradation of this endocannabinoid, as reflected by RT-PCR and immunohistochemistry. This decrease, again, was more marked in males than in females (Suárez et al., 2010). This sexual dimorphism was in agreement with our previous results showing that 13-day-old male rats were more affected than corresponding females regarding hippocampal neuronal and glial alterations (Llorente et al., 2008, 2009; López-Gallardo et al., 2008).

Moreover, two inhibitors of endocannabinoid inactivation (the fatty acid amide hydrolase inhibitor N-arachidonoylserotonin (AA-5-HT), and the endocannabinoid reuptake inhibitor, OMDM-2) modulated the above-indicated hippocampal cellular effects induced by the MD stress (Llorente et al., 2008). As a whole, these data support a clear association between neurodevelopmental stress and dysregulation of the ECS. This association may be relevant for schizophrenia and other neurodevelopmental psychiatric disorders. Moreover, we propose that the MD procedure may provide a relevant experimental model to further address the role of the ECS in brain development and its possible implications in neurodevelopmental mental illnesses such as schizophrenia. CB_2 receptors have been traditionally considered as peripherally located receptors, mainly expressed in immunological tissues. These receptors have also been found within the central nervous system on neurons and glial cells, but their expression was mainly related to conditions of inflammation. However, recent findings of brain CB_2 receptors under normal conditions suggest broader functional roles for these receptors in the central nervous system (for review see Mackie, 2008; Svizenska et al., 2008; Viveros et al., 2007). In our study on the effects of MD on cannabinoid receptors, we provided evidence for the presence of the CB_2 receptor in the hippocampus of neonatal rats of both sexes. Moreover, our data demonstrated that CB_2 receptors were clearly affected by the MD procedure: MD male and female animals showed a clear increase in CB_2 receptor immunoreactivity.

As we have seen, MD induces strikingly contrary patterns of changes on CB_1 and CB_2 receptors. The functional significance of such changes needs to be addressed, but suggests that there are functional links of some kind between both types of cannabinoid receptors with respect to the response to developmental challenges from the stress imposed by MD. Another difference regarding both receptors is that the CB_2 type was affected equally by MD in the two sexes, whereas CB_1 showed a clear sexual dimorphism with a more predominant decrease in the expression observed on MD males (Suarez et al., 2009). In view of recent reports on the presence of CB_2 receptors in diverse brain regions of adult rats and mice, and also considering the present findings, the functional roles of CB_2 receptors in the brain of naive rodents clearly deserve further investigation. Interestingly, there are several recent papers that suggest a role for CB_2 receptors in neuropsychiatric diseases (for review see Roche and Finn, 2010). For example, Ishiguro et al. (2010) described a polymorphism in the gene encoding for CB_2 receptors associated with schizophrenia in a Japanese population, and showed that administration of a CB_2 receptor antagonist worsened disruption of PPI induced by the NMDA receptor antagonist MK-801 in rats (Ishiguro et al., 2010). The potential implication of a role for the CB_2 receptor in schizophrenia provides another possible mechanism by which cannabis use could affect psychoses such as schizophrenia. Moreover, studies in mice overexpressing cannabinoid CB_2 receptors indicated that increased CB_2 receptor expression significantly reduced depressive-related behaviours, suggesting that the CB_2 receptor could be a new potential therapeutic target for depressive-related disorders (García-Gutiérrez et al., 2010).

Adolescence as a critical neurodevelopmental period

In addition to the perinatal period, adolescence represents another critical developmental phase (Figure 1(A)) during which the nervous system shows a unique plasticity. During this period, the brain undergoes radical functional alterations that are associated with a high degree of plastic structural remodelling (Giedd et al., 1999; Gogtay et al., 2004; Jernigan et al., 1991; Paus, 2005; Pfefferbaum et al., 1994; Shaw et al., 2006; Sowell et al., 1999). Areas involved in planning and decision-making, including the prefrontal cortex – the cognitive or reasoning area of the brain important for controlling impulses and emotions – appear not to have yet reached adult dimensions during the early twenties. The brain's reward centre, the ventral striatum, is more active during adolescence than in adulthood, and the adolescent brain is still strengthening connections between its reasoning- and emotion-related regions (Sowell et al., 1999). It has been proposed that adolescence involves a shift from greater limbic to PFC control of behaviour, with an increase in the inhibitory connections between these two regions (Spear, 2000). These neural changes are believed to underlie a shift from behaviour that is driven by affective impulses to more regulated behaviour that is guided by consideration of future personal and social consequences (Nelson et al., 2005). Therefore these findings suggest that cognitive control over high-risk behaviours is still maturing during adolescence, making teens more apt to engage in risky behaviours. In fact, adolescence is defined by characteristic behaviours that include high levels of risk taking, exploration, novelty and sensation seeking, social interaction, activity and play behaviours. The ages associated with adolescence are commonly considered in humans to be approximately 12 to 20–25 years of age, and PND 28–42 in rodents (Adriani and Laviola, 2004; Spear, 2000). A 'window of vulnerability' appears to exist during the periadolescent period regarding the onset of certain neuropsychiatric disorders such as schizophrenia and the effects of drugs of abuse, in particular cannabis (Adriani and Laviola, 2004; Fernandez-Espejo et al., 2009).

Sex-dependent long-term effects of adolescent exposure to cannabinoids

Although the rate of marijuana use among youths aged 12–17 has remained stable during recent years (6.7%), marijuana has been the illicit drug with the highest rate of dependence or abuse in recent years (SAMHSA, 2008). The increasing use of cannabis among adolescents and its associated public health problems have led to a parallel increase in basic research on appropriate animal models. Chronic administration of cannabinoid receptor agonists during the periadolescent period causes persistent behavioural alterations in adult animals. Some of these alterations may be related to a possible increased risk of psychosis and other neuropsychiatric disorders. As we will discuss in the next section, the early adolescent period is being identified as a phase of development particularly vulnerable to at least some of the adverse effects of exposure to cannabinoid compounds. For example,

Schneider and Koch (2003) showed that chronic pubertal treatment with the cannabinoid agonist, WIN 55,212-2, resulted in impaired memory in adulthood, whereas if the chronic treatment with the drug was administered during adulthood, it did not lead to behavioural changes (Schneider and Koch, 2003). In another study, a 21-day treatment with the cannabinoid receptor agonist CP 55,940 in 30-day-old rats resulted in a lasting impairment of working memory (O'Shea et al., 2004) and, again, these later behavioural changes were observed in adolescent but not adult drug-treated rats. A more recent study performed in male rats has shown that pubertal, but not adult, chronic WIN 55,212-2 administration induced persistent disturbances in object and social recognition memory (indicating impairments in working memory and social memory, respectively) and led to social withdrawal and alterations in social behaviour and self-grooming. Furthermore, acute administration of WIN 55,212-2 induced more severe effects on behavioural performance in pubertal than in adult rats (Schneider et al., 2008). Exposure of male rats to chronic THC caused greater lasting memory deficits and hippocampal alterations in adolescent than adult rats (Quinn et al., 2008). In support of these experimental data, early onset cannabis users (who began smoking before age 17) exhibit poorer cognitive performance than late-onset users (who began smoking at age 17 or later) or control subjects, especially in verbal IQ (Pope et al., 2003). On the other hand, O'Shea et al. (2006) found that chronic exposure to the cannabinoid agonist CP 55,940 during perinatal, adolescent or early adulthood induced similar long-term memory impairments in male rats. To explain the different results with respect to their previous study performed in female rats (O'Shea et al., 2004, see above), they claimed that adult males might be more vulnerable than adult females to some detrimental effects of cannabinoids, such as cognitive effects. In line with this proposal, we have recently shown that, in the novel object recognition test, males were more vulnerable than females to the detrimental effects of a protocol of chronic adolescent administration of CP 55,940 (Mateos et al., 2010). Our results also indicated that in the object location task, only the females showed a significantly impaired performance in response to adolescent cannabinoid exposure (PND 28–43), suggesting that diverse aspects of memory function may be differentially affected in each gender (Mateos et al., 2010). Rubino et al. (2009) showed that a subchronic treatment with THC from PND 35–45 resulted, in adulthood (PND 75), in a worse performance in the radial maze, although no alteration was found in aversive memory (passive avoidance) (Rubino et al., 2009). Thus, it seems that the long-term residual effects of adolescent chronic cannabinoid exposure are gender and task dependent. The duration and onset of the treatments are also important factors that may affect outcomes, but it seems clear that, in all cases, the effects of cannabinoids on cognitive function are deleterious and can be observed after a long wash-out period.

Another important point concerns the specific composition of the plant ('cannabis brands'). Recent data suggest that Δ-9-THC and cannabidiol (CBD), the two main ingredients of the *Cannabis sativa* plant, can have opposite effects on certain regional brain functions, which may underlie their different symptomatic and behavioural effects, and the potential ability of CBDs to somehow 'buffer' the detrimental (psychotogenic) consequences of THC (Bhattacharyya et al., 2010). A potential explanation for this interaction may be the recently described properties of CBD as an antagonist of CB_1 and CB_2 receptors (Thomas et al., 2007). In fact, the ratio of CBD and THC seems to have changed in an unfavourable manner in current 'cannabis brands'. This might underscore the higher risk of adverse (and long-lasting) consequences of marijuana consumption during adolescence. Nevertheless, more information is urgently needed in order to further clarify the extent to which CBD can in fact diminish the detrimental effects of THC, and which specific effects. A better understanding of the effects of CBD per se and in combination with THC requires more studies in which the drugs are administered chronically. This approach may also help to further clarify the potential therapeutic effect of CBD. In this regard animal models are a very useful tool.

Patterns of drug abuse have been recently reviewed (Greenfield et al., 2010). The rate of current illicit drug use is higher for males than for females. Accordingly, males are more likely than females to be past-month users of marijuana (7.9% vs. 4.4%). In spite of this fact, the rate of current use of marijuana among females has notably increased during recent years, while the rate has not changed significantly for males (SAMHSA, 2008). In agreement with findings in rodents, human studies also suggest the existence of gender differences as regards cannabis-induced cognitive impairment in young people (Pope et al., 1997), although much more research is necessary in evaluating sexual dimorphisms. It is very likely that the long-term cognitive effects of adolescent cannabinoid exposure are related to fewer synaptic contacts and/or less efficient synaptic connections throughout the hippocampus, and this could represent the molecular underpinning of the cognitive deficit induced by adolescent cannabinoid treatment (Rubino et al., 2009). Moreover, it is tempting to speculate that a differential effect on synaptic plasticity could be found depending on the gender of the animals in parallel with differential behavioural impact. A possible link between the impaired memory observed specifically in males pre-exposed to cannabinoids in the novel object test and the increased functional activity of their CB_1 receptors (Mateos et al., 2010) might be the CB_1-mediated inhibition of glutamatergic and GABAergic neurons involved in mnemonic circuits (Ferraro et al., 2009; Larkin et al., 2008; Viveros et al., 2007).

In addition to cognitive effects, other cannabinoid effects have been also shown to be sexually dimorphic. For instance, we addressed the behavioural features of adult rats which had been exposed to chronic treatment with CP 55,940 (0.4 mg/kg) during the juvenile period (from 35–45 days of age). We used a battery of tests which provide complementary data about diverse aspects of the spontaneous behaviour of the animals and their anxiety-related responses. In the holeboard test, CP 55,940-treated females showed decreased general motor activity, and a significantly increased head-dipping duration (an exploratory parameter). In contrast, males treated with CP 55,940 in the above-described juvenile period showed a significant decrease in exploratory activity, whereas their general motor activity was not modified. Our results also indicated that the animals treated with CP 55,940 in youth

(days 35–45) showed anxiolytic-like responses in adulthood, as measured in the plus-maze and in an illuminated open field (Biscaia et al., 2003). However, the effects on anxiety-related responses appear to be dependent on the duration of the pharmacological treatment, and perhaps the test employed, since other authors, using different protocols and/or test of anxiety have reported long-term increases in anxiety as a result of adolescent cannabinoid exposure (Viveros et al., 2005a). As for other types of emotional response, Rubino et al. (2008) demonstrated that chronic administration of THC in adolescent rats induced subtle but lasting alterations in the emotional circuit ending in depressive-like behaviour in adulthood, and that this effect was observed in female but not in male rats.

Chronic peripubertal cannabinoid exposure: an animal model for specific signs of psychosis

Cannabis is one of the most abused drugs among teenagers, and the maturational processes that occur during adolescence are likely to confer this age group a higher risk of suffering from adverse consequences of cannabinoid exposure. Cannabis consumption has been related to detrimental emotional and cognitive consequences. In particular, a great health concern has arisen given its association with depression (Degenhardt et al., 2003) and with an increased risk of psychosis (Di Forti et al., 2009; Fernandez-Espejo et al., 2009). In this section, we will focus on cannabinoid-induced neuropsychiatric disorders, with special emphasis on psychotic symptomatology.

Based on a large amount of data from animal studies, an association between early and/or heavy chronic cannabinoid use and detrimental long-term consequences to those at risk for schizophrenia has been concluded. Prospective longitudinal epidemiological studies may provide evidence for a higher incidence of neuropsychiatric disorders in participants who have consumed cannabis during adolescence. However, the ultimate proof of a causal relationship between cannabis use and psychotic illness later in life would come from studies in which healthy young people were exposed to THC and followed-up until adulthood. Obviously, for practical and ethical reasons, such an approach is impossible. In fact, among many other important health risks, it is well known that cannabis induces harmful effects on cognitive function (Nordentoft and Hjorthoj, 2007; Solowij and Michie, 2007; Solowij et al., 2002). On the other hand, such studies can be performed in animals under well-controlled conditions. Hence, such animal models can shed light on the underlying neurobiological mechanisms, and on the relationship between cannabis use and schizophrenia. A dysregulation of the ECS may be implicated in the pathogenesis of schizophrenia. The peripubertal period appears to be critical for the development of cannabinoid CB_1 receptors and endocannabinoid levels (Rodriguez de Fonseca et al., 1994; Wenger et al., 2002). Therefore, it is conceivable that chronic interference by cannabis with the developing ECS during this critical time interval leads to severe and persistent functional impairments (Schneider and Koch, 2007) that might reflect, at least in part, psychosis-related symptoms. Adolescent animal models have proven to be useful in analysing the association between adolescent cannabis use and the long-lasting development of psychotic symptoms. For example, chronic pubertal treatment with the cannabinoid agonist, WIN 55,212-2, resulted in impaired memory in adulthood as well as in a disrupted PPI of the acoustic startle response and lower breakpoints in a progressive ratio operant behaviour task (Schneider and Koch, 2003). Since PPI deficits, object recognition memory impairments, and anhedonia/avolition are among the endophenotypes of schizophrenia, the authors of this study proposed chronic cannabinoid administration. More recently it was confirmed that chronic pubertal WIN 55,212-2 treatment induced a long-lasting PPI deficit in adult rats as well as persistent changes of neuronal activity assessed by c-Fos protein quantification in several brain regions under basic conditions and in response to dopaminergic drugs (Wegener and Koch 2009). Interestingly, chronic WIN 55,212-2-treated rats not only showed a higher baseline Fos IR in the NAcc, a key structure of the mesolimbic reward system, but within this region also responded differently to dopaminergic drugs. The change in neuronal activity may represent a neuronal correlate for the effects of pubertal WIN 55,212-2 exposure on behavioural alterations observed during adulthood, possibly affecting the adult organism's response to certain drugs of abuse (Wegener and Koch, 2009). As in the vast majority of these kind of studies, this one was performed in male rats. In the next section we will see that in fact adolescent exposure to cannabinoids results in increased responses to other drugs of abuse, with this effect showing clear sexual dimorphisms.

The CB_1 agonist CP 55,940 has been reported not only to impair PPI in rats but also auditory gating and neuronal synchrony in limbic areas such as the hippocampus and entorhinal cortex, as evaluated through theta field potential oscillations (Hajos et al., 2008). It seems clear that, at least in rats, cannabinoid agonists impair auditory gating function in the limbic circuitry, supporting a connection between cannabis abuse and schizophrenia as evaluated through this animal model. As a whole, the data described above indicate that chronic pubertal cannabinoid treatment in rats results in long-lasting behavioural alterations reflecting certain characteristics of schizophrenia symptomatology, such as deficits in sensorimotor gating, impaired memory, reduced motivation and inappropriate and deficient social behaviour. In addition, sensorimotor gating deficits were able to be restored by acute injections of the typical antipsychotic haloperidol. Moreover, the atypical antipsychotic drug quetiapine is able to acutely restore deficits in social behaviour induced by developmental cannabinoid exposure, and even exert some persistent beneficial effects. All these data provide support and validity for the suitability of chronic pubertal cannabinoid administration as an animal model for aspects of psychosis and schizophrenia (Leweke and Schneider, 2011; Malone et al., 2010). It would be very interesting to directly address possible sexual dimorphisms regarding increased risk of showing schizophrenic-like symptoms in adolescent animals exposed to cannabinoids.

Interactions between cannabinoids and other drugs of abuse

It is well known that, in adult rodents, cannabinoid receptor agonists induce biphasic effects on anxiety (Viveros et al., 2005b) that may depend on the dose and the action on amigdalar–hypothalamic circuits involving corticotropin releasing factor (Rodriguez de Fonseca et al., 1996). However, when we evaluated the effects of the same cannabinoid agonist on the anxiety-related responses (plus-maze) of young 40-day-old male and female rats, the data indicated a different profile, suggesting that the anxiolytic response may be dependent on age. The most relevant result of these experiments was the lack of anxiolytic-like effects of CP 55,940, even at very low doses (0.1 mg/kg). On the other hand, the cannabinoid agonist induced anxiogenic-like effects at doses of 0.1 and 0.5 mg/kg, and females appeared to be more vulnerable than males to the anxiogenic effect of the drug (Viveros et al., 2005a). Nicotine and cannabis, which share some biological actions (including biphasic effects on anxiety), are used frequently in combination, particularly among adolescents and young adults, and therefore the study of their functional interactions is of special interest (Viveros et al., 2006). We were interested in the interactions of these drugs regarding possible synergistic or antagonistic effects in relation to anxiety, as this may help to understand one possible reason for, and consequences of, the simultaneous use of the two drugs. For example, students reported that they smoked tobacco to reduce the sedative effects of cannabis and to increase and prolong the rewarding effects of cannabis (Tullis et al., 2003). We addressed the effects of a subchronic treatment with nicotine upon acute anxiety-like responses to the cannabinoid receptor agonist CP 55,940 in adolescent rats of both genders. In males, the combination of sub-threshold doses of the two drugs resulted in a significant anxiogenic-like effect. On the other hand, females appeared to be more vulnerable to the anxiogenic effect of the cannabinoid, and this effect was antagonized by nicotine (Marco et al., 2006). The observed sexual dimorphism suggests that the combined use of nicotine and cannabis may have a very different effect on the emotional status of male and female adolescents, which might influence the pattern and motivations of its consumption.

According to the phenotypic causation (gateway) model, early initiation of cannabis use may be a risk factor for the consumption of other drugs of abuse (Lynskey et al., 2003), although the alternative 'correlated liabilities' model proposes that cannabis and other illicit drug use is influenced by correlated genetic and environmental factors (Agrawal et al., 2004). Here also, the use of animal models has been very useful in analysing possible neurobiological substrates for these interactions. Sex-specific patterns of consumption during all phases of drug addiction are well documented (for a review see Fattore et al., 2009). However, most of the experimental research in the field of drug abuse has been carried out on males. Ellgren et al. (2007), in a study performed on male rats, demonstrated that exposure to THC in adolescent animals produced an increase in heroin self-administration, preproenkephalin mRNA expression and the expression of met-enkephalin and mu-opioid receptors in adulthood. More recently we examined whether chronic periadolescent exposure to the cannabinoid agonist CP 55,940 (0.4 mg/kg, PND 35–45) could exert sex-dependent effects on morphine self-administration and the endogenous opioid system in adult rats. Periadolescent cannabinoid exposure altered morphine self-administration and the opioid system in adult rats in a sex-dependent manner. CP 55,940 did increase the acquisition of morphine self-administration under the FR1 schedule in males but not females. In addition, adolescent CP 55,940 exposure decreased mu-opioid receptor functionality in the NAcc shell only in males (Biscaia et al., 2008). According to our results, decreased mu-opioid-coupled G-protein activity occurred in the NAcc shell of male rats exposed prenatally to THC, with no changes in the NAcc core or caudate putamen (Spano et al., 2007). Together, these data suggest that cannabinoid exposure in early stages of development and adolescence produces perdurable changes in mu-opioid receptor functionality that are specific to the NAcc shell, which is one of the brain regions most closely related to natural and drug-induced reward (Di Chiara, 2002). The direction of sex differences regarding long-lasting effects of adolescent cannabinoid exposure on self-administration of other drugs of abuse may depend on the specific nature of the drug. By using a similar (though not identical) protocol as the one employed regarding the effects of adolescent CP 55,940 on morphine self-administration (Biscaia et al., 2008), Higuera-Matas et al. (2008) analysed the long-term effects of a chronic treatment with the same dose of CP 55,940 during adolescence (0.4 mg/kg, P28–P38) on adult acquisition and maintenance of cocaine self-administration. During the acquisition phase, female CP 55,940-treated rats showed a higher rate of cocaine self-administration as compared with vehicle-treated females and males, whereas no differences were found between both male groups (Higuera-Matas et al., 2008).

Mechanisms underlying gender differences: brain sexual differentiation

The original organizational–activational hypothesis of brain sexual differentiation has inspired a multitude of experiments demonstrating that the perinatal period is a time of maximal sensitivity to gonadal steroid hormones. According to this hypothesis, exposure to steroid hormones early in development masculinizes and defeminizes neural circuits (structural changes), programming behavioural responses to hormones in adulthood. Upon gonadal maturation during puberty, testicular and ovarian hormones act on previously sexually differentiated circuits to facilitate expression of sex-typical behaviours (activational effects) (Handa et al., 2008; Schwarz and McCarthy, 2008). Recent data have shed new insights into the mechanisms underlying sex differences. Thus, it has been proposed that the adolescent brain, undergoing remodelling, is organized a second time by gonadal steroid hormones secreted during puberty. This second wave of brain organization would build on and refine circuits that were sexually differentiated during early neural development. If, in fact, steroid-dependent organization of behaviour occurs during adolescence, this prompts a reassessment of the developmental time-frame within which organizational effects

are possible (Schulz et al., 2009). As for the sexual dimorphisms affecting the ECS and its diverse psychophysiological implications (for a review see Viveros et al. 2010), the number of carefully controlled studies designed to assess the organizational (i.e. in utero, neonatal) and/or activational (i.e. acute) roles of gonadal steroids in initiating and maintaining the disparities is limited. This is particularly problematic for human studies, where developmental, circadian and/or menstrual changes in the hormonal milieu are rarely, if ever, accounted for in the proper way.

In animal studies, endocrine status can be more precisely controlled. We showed that cannabinoid CB_1 receptor density in the medial basal hypothalamus, and expression in the anterior pituitary, varies over the course of the oestrous cycle (Rodriguez de Fonseca et al., 1994) – with the lowest levels observed during oestrus – and, in the case of the latter, is higher in males than in females and is increased following ovariectomy in an oestrogen-reversible manner (Gonzalez et al., 2000). This also appears to be the case with regard to cannabinoid self-administration, as gonadally intact female rats exhibit a higher rate of acquisition and maintenance than gonadally intact males, while ovariectomy reduced the levels of self-administration (Fattore et al., 2007). This suggests that CB_1 receptor expression is sexually differentiated in a regionally specific way, and subject to acute activational suppression by oestrogen. While there is clearly a prevalence of sex/gender differences in diverse biological processes regulated by cannabinoids, more work needs to be done in order to further our understanding of how gonadal steroids organize these disparities during in utero and neonatal development, and maintain them throughout life. The ideal way to test for sex differences in a given cannabinoid effect is to perform direct comparisons between castrated male and female subjects.

Concluding remarks

Animal models have clearly demonstrated that the ECS is a relevant contributor to brain development. Disruption of its functioning in critical periods may lead to silent phenotypes of vulnerability to mental health problems. These alterations may be derived from genetic disruptions or environmental modulations that may lead to epigenetic alteration of the ECS, as revealed by early stress-induced alterations in methylation patterns of the cannabinoid CB_1 gene (Franklin et al., 2010). This work clearly shows how well-known developmental factors that account for enhanced incidence of major mental disorders may also modulate the ECS, or even produce their impairment through this signalling system.

The MD model has been shown to be instrumental in establishing this relevant role for the ECS. The importance of the ECS as a key target for epigenetic factors stresses the importance of controlling cannabis exposure during critical developmental periods, mainly in adolescence.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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