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Framework for sex differences in adolescent neurobiology: A focus on cannabinoids

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Abstract

This review highlights the salient findings that have furthered our understanding of how sex differences are initiated during development and maintained throughout life. First we discuss how gonadal steroid hormones organize the framework for sex differences within critical periods of development—namely, during those exposures which occur *in utero* and post-partum, as well as those which occur during puberty. Given the extensive precedence of sex differences in cannabinoid-regulated biology, we then focus on the disparities 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. 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, followed by the organizational and activational roles of gonadal steroids in establishing and maintaining the sex dependence in the biological actions of cannabinoids. Finally, we discuss ways to utilize this knowledge to strategically target critical developmental windows of vulnerability/susceptibility and thereby implement more effective therapeutic interventions for afflictions that may be more prevalent in one sex vs. the other.

Keywords

Periadolescent period; Sex differences; Cannabinoids; Organizational and activational effects; Gonadal steroids

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1. Introduction

The transition from childhood to adult life is accompanied by prominent structural and functional modifications in the central nervous system, which are associated with modifications in cognition, mood, sleeping patterns, personality, social interactions, behavior and affection. Adolescence is a period of an intense growth, reshaping and maturation of the grey and white matters in the human brain, with marked regional and sex differences. In general, cortical grey matter thickness peaks earlier in females than in males and some sex differences in brain structure are generated during pubertal maturation (García-Segura, 2009). Some of the structural and functional plastic changes that occur in the central nervous system during adolescence are hormonally regulated. The functional consequences of many early hormonal effects on brain organization are manifested during adolescence. Pre-natal and early postnatal neuroplastic regulatory actions of sex and stress hormones may predetermine future metaplastic modifications in several brain regions during adolescence. Indeed, the adolescent brain is highly sensitive to actions of sex and stress hormones. Therefore, hormonal actions during this life stage may cause additional long-term modifications in the pattern of synaptic plasticity, affection, cognition and behavior (García-Segura, 2009; Spear, 2004) that may last throughout life. Since specific aspects of adolescent behaviors are shared across species, many questions about adolescent brain development and its impact on disease can be investigated in animal models. In particular, adolescent rats exhibit a particular behavioral repertoire that resembles human adolescent behavior (Adriani and Laviola, 2004; Crews et al., 2007; Laviola et al., 2003; Spear, 2000). In the first part of this review, we will first refer to the main characteristics of adolescence as a critical period for brain development and behavioral maturation. We will then analyze the critical periods for brain sexual differentiation during which the action of gonadal hormones on diverse brain circuits may establish many of the sex differences observed throughout the psychobiology of both humans and experimental animals.

Cannabis is one of the most abused drugs among teenagers and the maturational processes that occur during adolescence are likely to confer on this age group a higher risk of suffering from adverse consequences of cannabinoid exposure (SAMHSA, 2008). Recent studies have unequivocally documented the occurrence of a cannabis withdrawal syndrome (Budney et al., 2004; Fattore et al., 2008; Tanda and Goldberg, 2003). This cannabis withdrawal syndrome seems to be characterized by craving, irritability, anxiety, depressed mood, decreased appetite and sleep difficulties (Vandrey et al., 2008). Cannabis consumption has also been related with 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). Adolescence appears to be a critical period for the development of cannabinoid CB_1 receptors and endocannabinoid levels. Therefore, it is conceivable that chronic interference by exogenous cannabinoids with the developing endocannabinoid system during this critical time-interval leads to severe and persistent functional impairments. Both human and animal studies indicate that cannabis use during adolescence may produce cognition impairments and depressive symptoms and may increase the risk to develop substance abuse disorders and psychotic-like symptoms (Marco and Viveros, 2009; Realini et al., 2009; Schneider,

2008, 2009; Viveros et al., 2005a). 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. In the second part of this paper we will focus on diverse psychobiological aspects of the endocannabinoid system during the adolescent period and will pay special attention to the possible mechanisms underlying the numerous sexual dimorphisms affecting this important neuromodulatory system.

Research on sex differences in this respect has increased in the last years, though there are still scarce systematic studies including both genders in humans, preclinical information has provided some clues about the possible underlying mechanisms for observed sexual dimorphisms. In fact, chronic administration of cannabinoid agonists during adolescence causes long-term neurochemical and behavioral alterations, including cognitive deficit and substance addiction, with numerous sexual dimorphisms becoming evident in adult animals. Moreover, interactions between cannabinoids and other drugs of abuse also show clear sex differences in relation to anxiety- and depressive-like responses as well as addiction-related processes. In the last section of this manuscript we will discuss organizational vs. activational effects of gonadal steroid hormones in the establishment and maintenance of sex differences in the psychobiological actions of cannabinoids. Herein we present data that highlight the urgent need for researchers to contemplate sex differences along the lifespan, and to consider the developmental effects of gonadal steroids in their approach to the study of general sex differences.

2. Framework for gender differences in adolescent neurobiology

2.1. Adolescence as a critical neurodevelopmental period

The transition from childhood to adulthood is a critical period of transformation in the life cycle. Although mature gonadal function in vertebrates is reached at puberty, the full process of transition from childhood to adulthood involves a progressive maturation that is initiated before puberty and that is not entirely completed by the time of puberty. In this review we will consider adolescence as the gradual period of transition from childhood to adulthood, including pubertal maturation.

Adolescence is a critical phase in the maturation of the central nervous system. This period of neural maturation involves neurocognitive, hormonal and psychosocial changes with considerable modifications in cognition, mood, arousal, motivation, sleeping patterns, personality, social interactions, behavior and affection in humans (Blakemore, 2008; Giedd, 2008; Steinberg, 2005) During this period, the brain undergoes radical functional alterations that are associated with a high degree of plastic structural remodeling (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). Different brain regions have different peaks of maturation and the changes include modifications in the volume of grey and white matter. Brain areas related to more basic functions (instinctive), including the primary sensorimotor cortices, mature early whereas in associative cortical regions involved in the integration of information from different sensory modalities, such as the temporal lobes, grey matter volume does not reach adult steady state until the early to mid twenties (Giedd et al., 1999; Gogtay et al., 2004). 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 dimension during the early twenties. The brain's reward center, the ventral striatum is more active during adolescence than in adulthood, and the adolescent brain still is 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 prefrontal cortical (PFC) control of behavior, with an increase in the inhibitory connections between these two regions (Spear, 2000). These neural changes are believed to underlie a shift from behavior that is driven by affective impulses to more regulated behavior 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 behaviors is still maturing during adolescence, making teens more apt to engage in risky behaviors. In fact, adolescence is defined by characteristic behaviors that include high levels of risk-taking, high exploration, novelty and sensation-seeking, social interaction, high activity and play behaviors. 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). Adolescent behaviors are shared across species, for example, adolescent rodents exhibit a particular behavioral repertoire that resemble human adolescent behavior including high levels of exploration, novelty and sensation-seeking, impulsivity and an increased sensitivity to incentives. These behaviors have been suggested to help adolescents develop the social skills needed when they become independent from their family or become senior adults in their group. On the other hand, with the brain's emotion-related areas and connections still maturing, adolescents may be more vulnerable to psychological disorders. The high levels of novelty/sensation-seeking behaviors appear to be strong predictors of drugs use among adolescents (see for review: Adriani and Laviola, 2004; Crews et al., 2007; Laviola et al., 2003; Spear, 2000).

2.2. Sex differences in brain maturation during adolescence

Numerous structural sex differences in the brain are manifested during adolescence. In humans, the rates of brain growth show sex differences in some brain regions, such as the cerebral cortex, the hippocampus, the amygdala, the bed nucleus of the stria terminalis and the corpus callosum. Thus, the age of peak of cortical grey matter thickness occurs in general earlier in girls than in boys accordingly with the earlier puberty onset in girls (De Bellis et al., 2001; Giedd, 2004; Giedd et al., 1997). In contrast, boys had more prominent age-related white matter volume and corpus callosal area increases compared with girls (De Bellis et al., 2001). Furthermore, some sex differences in brain structure are generated during adolescence, such as the differences in volume and number of neurons in the bed nucleus of the stria terminalis, which is larger in men (Chung et al., 2002).

Animal studies have revealed marked changes in brain structure during the transition from childhood to adulthood. Axons, dendrites, synapses and synaptic receptors are modified in several brain regions between puberty and adulthood (Sisk and Zehr, 2005). In general, studies in animals are in agreement with the observations in humans, indicating that there is a reorganization of neuronal connectivity in the grey matter after puberty (Anderson et al., 1995; Meyer et al., 1978; Yildirim et al., 2008). In addition, the pattern of neuronal/dendritic proliferation varies with the cortical/neocortical region (i.e., medial prefrontal cortex,

hippocampus) being examined in a sex-dependent manner (Juraska et al., 1985, 1989; Markham et al., 2007). The morphological remodeling is accompanied by significant modifications in the expression of synaptic proteins such as spinophilin and synaptophysin (Zehr et al., 2006). Major neurotransmitter systems are not mature at birth and postnatal brain development continues through adolescence, with remodeling most pronounced in frontal and limbic regions (see Crews et al., 2007). Biochemical analyses have revealed modifications in the expression of synaptic receptors. The expression of striatal dopamine receptors show marked sex differences during adolescence. Males show a much higher increase than females during adolescence and a much higher decrease in adulthood (Andersen and Teicher, 2000). It has been shown that dopamine (DA) D_1/D_2 receptor density in rat striatum and prefrontal cortex peaked at the onset of puberty and declined by 58–75% upon reaching adulthood (Andersen et al., 2000; Teicher et al., 1995). The reorganization of serotonin (5-HT) receptor expression is also pronounced during development, likely relating to reorganization of serotonergic innervation patterns. For example, 5-HT_{2A} receptors reach cortical peak expression just before adolescence and then progressively decline to adult levels correlated with increased innervation and pruning of 5-HT axons (Morilak and Ciaranello, 1993). 5-HT turnover in NAc is reported to be approximately 4-fold lower in adolescent rats (PND 30–40) than either younger rats (PND 10–15) or mature adults (PND 60–80) (Teicher, 1999). All these findings indicate that the transition from childhood to adulthood is accompanied by a reorganization of synaptic connectivity which may be associated with new behavioral performances. For example, during the prepubertal period there is a maturation of interactions between D_1 receptors and NMDA receptors in the prefrontal cortex that may be critical for developing mature cognitive abilities (Tseng and O'Donnell, 2005). Sex dimorphic changes have been detected in neurons from the preoptic area of adolescent macaque monkeys, where juvenile males have more dendritic bifurcations and a higher frequency of spines than juvenile females (Ayoub et al., 1983) as well as in the volume and number of neurons have also been reported in specific brain regions during adolescence in rodents (Markham et al., 2007; Nunez et al., 2001; Reid and Juraska, 1992). These changes may be the consequence of a different rate of neuronal death during the transition from childhood to adulthood. For instance, in rats there is a sexually dimorphic increase in cell death in the visual cortex during adolescence (Nunez et al., 2001), resulting in adult male rats having 19% more neurons than female rats in the binocular region and 18% more in the monocular region of the primary visual cortex (Reid and Juraska, 1992). The volume and the number of neurons of the ventral portion of the prefrontal cortex are also reduced in female rats but not males between days 35 and 90 (Markham et al., 2007).

Another cause for the generation of sex differences in the number of neurons is the generation of new cells during adolescence. Changes in the rate of neurogenesis also occur during adolescence. It has been detected that new cells, including neurons, are incorporated to different brain regions during adolescence in rats. These brain regions include the anteroventral periventricular nucleus of the hypothalamus, the sexually dimorphic nucleus of the preoptic area and the medial amygdala (Ahmed et al., 2008). In addition, there is a decrease in levels of neurogenesis in the subgranular layer of the hippocampal dentate gyrus and in the forebrain subventricular zone during adolescence (Crews et al., 2007). It is

unknown whether this also occurs in many other brain regions where sex differences in the number of neurons and glial cells have been detected, including the cerebral cortex.

2.3. Hormonal regulation of brain plasticity during adolescence

Gonadal hormones appear to be important regulators of neuroplastic modifications during adolescence, in which sex differences in brain maturation are observed (De Bellis et al., 2001; Giedd, 2004; Giedd et al., 1997; Markham et al., 2007; Nunez et al., 2001). A central tenet of contemporary theories on mammalian brain and behavioral sexual differentiation is that an organizational action of testosterone, secreted by the male's testes, controls male-typical aspects of brain and behavioral development whereas no active perinatal sex hormone signaling is required for female-typical sexual differentiation. However, recent data demonstrate that estradiol actively contributes to the differentiation of female-typical aspects of brain and behavioral sexual differentiation (Bakker and Brock, 2010).

2.3.1. Testosterone—The sexual differentiation of the central nervous system is a process involving several critical periods. These critical periods differ between species and correspond with surges in plasma testosterone during development. In rats and mice the main critical period spans from late gestation to early postnatal development, in guinea pigs between days 30 and 37 of gestation, in sheep from 30 to 147 days of pregnancy and in rhesus monkeys and humans is in the first trimester of pregnancy (Resko and Roselli, 1997; Robinson, 2006). In addition, the organizational effect exerted by testosterone during these early critical periods may be refined and reshaped by further hormonal actions, latter on, during adolescence (Davis et al., 1996; Robinson, 2006).

Testicular hormones are responsible for both the increase in the density of dendritic spines in hippocampal neurons of male rats during puberty and for their decrease after puberty, since these plastic modifications are prevented by castration before puberty (Meyer et al., 1978). Testicular hormones are also associated with functional modifications in synaptic plasticity in the hippocampus during puberty. Thus, Hebbard et al. (2003) have shown that male rats that were gonadectomized at the onset of puberty and treated with testosterone during puberty showed a shift from long-term synaptic potentiation toward long-term synaptic depression in the CA1 region of the hippocampus and a reduction of social memory. Testosterone also regulates dendritic formation and dendritic pruning in the spinal nucleus of the bulbocavernosus (Goldstein et al., 1990), promoting the initial growth of dendrites. Furthermore, the increase in testicular hormones with puberty appears to prevent further dendritic retraction, stabilizing dendritic length at the adult levels (Goldstein et al., 1990).

Testicular hormones regulate as well the incorporation of new cells to specific brain regions during adolescence in rats (Ahmed et al., 2008). Some of the neuroplastic modifications exerted by testicular hormones during the transition from childhood to adulthood are influenced or pre-programmed by early hormonal actions. For instance, the sexual dimorphism in the volume of the anteroventral periventricular nucleus of the rat hypothalamus, which is larger in females than in males, arises between postnatal days 30 and 40. Then, the length of the nucleus becomes sexually dimorphic between days 60 and 80. However, the appearance of this sexual dimorphism is dependent on perinatal levels of

androgens, since castration of male rats on the day of birth sex-reversed anteroventral periventricular nucleus volume in adulthood and anteroventral periventricular nucleus length was sex-reversed by castration of males 5 days after birth (Davis et al., 1996). These findings suggest that prenatal and early postnatal regulation of brain mutability by testosterone may predetermine some of the structural and functional plastic changes that these hormones may regulate latter on, during the peripubertal period. Another example is the sex difference in volume of the locus coeruleus of rats, which is due to a greater number of neurons in females. The number of neurons progressively increases in the locus coeruleus in both sexes; however, postpubertal females have more neurons in the locus coeruleus than postpubertal males probably by a sex difference in neuronal migration or neurogenesis around the time of puberty (Pinos et al., 2001). Interestingly, sex difference in the number of neurons in the locus coeruleus depends on perinatal levels of androgens but, as for the anteroventral periventricular nucleus, it is manifested during puberty.

2.3.2. Ovarian hormones—Estradiol is also involved in the regulation of brain plasticity during the transition from childhood to adulthood. Despite the current dogma states that the female brain develops independently of estradiol, many studies have hinted at possible roles of estrogen in female sexual differentiation. Indeed, many, though not all, of the perinatal organizational actions of testosterone on the development of the male brain actually results from the cellular effects of estradiol formed via neural aromatization of testosterone. It has been suggested that male-typical neural and behavioral differentiation occurs prenatally in genetic males under the influence of estradiol which is avoided in fetal genetic females by the neuroprotective actions of alpha-fetoprotein, whereas female-typical neural and behavioral differentiation normally occurs postnatally in genetic females under the influence of estradiol presumably produced by the ovaries (Bakker and Baum, 2008; Bakker and Brock, 2010).

Estrogens play an important role in developmental synaptogenesis through diverse mechanisms, including glycocalyx glycoproteins in neuronal membranes, neural cell adhesion molecules, and insulin-like growth factor I. An important relationship between circulating estrogen, gonadotropin levels, and hypothalamic synaptic plasticity has been demonstrated, possibly mediated by GABAergic and dopaminergic synaptic inputs and POMC projections from the arcuate nucleus to the GnRH cells (Naftolin et al., 1996). Moreover, estradiol increases dendritic and soma spine density in the hypothalamic ventromedial nucleus in juvenile and peripubertal male and female rats (Segarra and McEwen, 1991). In addition, the decrease in neuronal numbers in the visual cortex of female rats during puberty is prevented by pre-pubertal ovariectomy, suggesting that ovarian hormones are the main regulators of this developmental change in the cerebral cortex (Nunez et al., 2002). A modification in the regulation of IGF-I receptor signaling by estradiol during pubertal maturation in the prefrontal cortex of female rats may be involved in the plastic modifications occurring in this brain region (Sanz et al., 2008).

Actions of estradiol during the peripubertal period are also involved in the differentiation of the pattern of axonal connectivity of neurokinin B expressing neurons in the rat hypothalamic arcuate nucleus. Neurokinin B expressing neurons project their ventral axons to capillary vessels in males and to the neuropil in females. This sexually dimorphic pattern

of axonal projections is apparently regulated by a masculinizing action of testosterone, via androgen receptors, and a feminizing action of estradiol at puberty (Ciofi et al., 2007). Ovarian hormones during the peripubertal period are also involved in the regulation of the connectivity between the two brain hemispheres, since ovariectomy on postnatal day 20 results in an increase in the number of myelinated axons in the splenium of the corpus callosum of young adult rats. Ovarian hormones seem to affect the process of myelinization but the total number of axons is not affected by prepubertal ovariectomy (Yates and Juraska, 2008). Ovarian hormones also regulate the incorporation of new cells to some brain regions during adolescence in rats (Ahmed et al., 2008).

2.3.3. Stress hormones—Another important hormonal influence for brain plasticity during the transition from childhood to adulthood is provided by stress hormones. The adolescent brain is highly sensitive for the plastic actions of stress hormones (Ferris, 2000), which exert effects that will be transmitted to adulthood. The physiological and behavioral responses to stress are modified during adolescence, which is the moment when the full functional maturation of the hypothalamic-pituitary-adrenal axis occurs (Di Luigi et al., 2006; Goel and Bale, 2007; Meaney et al., 1985; Romeo et al., 2004, 2006). This is also a period in which previous stressful experiences of early life may impact on brain plasticity and function. For instance, early stress has some effects on synaptic plasticity that are not manifested until adolescence. One example is maternal deprivation, which causes a disruption of prepulse inhibition of the startle response in rats that is only detected after puberty (Ellenbroek et al., 1998). Interestingly, alterations in prepulse inhibition also occur in schizophrenia and the manifestation of the disruption of prepulse inhibition after puberty in maternally deprived rats coincides with the temporal profile for the onset of schizophrenic symptoms in humans. Early stress also interferes with the maturation of interneurons in the medial prefrontal cortex of rodents at the age around puberty (Helmeke et al., 2008). In some cases, interactions with developmental and/or peripubertal actions of sex hormones occur. Thus, neonatal handling increases stress-induced activation of the posterior cingulate cortex of adolescent female rats and not of adolescent males (Park et al., 2003). In contrast, cognitive deficits and behavioral alterations as a consequence of prenatal stress have been detected in adolescent male rats and not in adolescent females (Nishio et al., 2001). In addition, stress during adolescence may also have different effects in male and female brains. For instance, stress during adolescence enhances locomotor sensitization to nicotine in adult female rats, but not in adult male rats (McCormick et al., 2004).

3. A focus on cannabinoid biology

Sex/gender differences are pervasive throughout the animal kingdom. They are found at every level of biology—from the organismal to the molecular. Due to the organizational and activational effects of gonadal steroid hormones, sex differences in vertebrates are often forged before birth, and persist throughout an individual’s lifespan. While there are many different kinds of sex differences that could be discussed in this review, we have chosen to focus on those pertaining to cannabinoids as an example to explain the urgent need for considering the developmental effects of gonadal steroids in their approach to study sex differences. There exists a plethora of examples of sex/gender differences in cannabinoid-

regulated biology. These include, but are not limited to, differences in delta-9-tetrahydrocannabinol (THC) metabolism (Narimatsu et al., 1991; Watanabe et al., 1992), as well as in cannabinoid-mediated antinociception (Tseng and Craft, 2001), locomotor changes (Tseng and Craft, 2001; Wiley, 2003), hemodynamics (Mathew et al., 2003), impairment of visuospatial memory (Pope et al., 1997), self-administration (Fattore et al., 2007), food/energy intake (Farhang et al., 2009; Miller et al., 2004), and neurotransmission at proopiomelanocortin (POMC) synapses in the hypothalamic arcuate nucleus (ARC) (Farhang et al., 2009). In the following sections we will focus specifically in sexual dimorphisms affecting cannabinoid-mediated biological processes during critical developmental periods, with a particular emphasis in the adolescence.

3.1. Developmental aspects of cannabinoid CB_1 receptors

The CB_1 cannabinoid receptor is a key component of the endocannabinoid system (ECS) which consists of endogenous ligands called endocannabinoids, typically anandamide (AEA) and 2-arachidonylglycerol (2-AG), which act upon activation of cannabinoid receptors (types 1 and 2, CB_1 and CB_2 receptors, respectively). 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). Among the multiple functions of the endocannabinoid system (Bermudez-Silva et al., 2010; Cota, 2008; Moreira and Lutz, 2008; Viveros et al., 2005b, 2007; Wotjak, 2005) there is evidence for its role in neural development. 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). During the perinatal period, a common atypical pattern of CB_1 receptors 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 and atypical pattern of CB_1 receptors localization in white matter areas during the prenatal stages, suggests a specific role of the endocannabinoid system in neural development (Belue et al., 1995; Mato et al., 2003; Rodriguez de Fonseca et al., 1993). At early developmental stages, the ECS seems to influence the expression of key genes for neural development, to participate in axonal growth and fasciculation and in the establishment of correct neuronal connectivity (Fernandez-Ruiz et al., 2004; Harkany et al., 2007; Watson et al., 2008). 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 have been shown to mature slowly, with maximal levels during adolescence which later drop to adult levels (Belue et al., 1995; Rodriguez de Fonseca et al., 1993). Thus, as for other major neurotransmitter systems (e.g., dopaminergic (Andersen et al., 2000; Teicher et al., 1995), serotonergic (Morilak and Ciaranello, 1993; Teicher, 1999), etc., see previous section for more details), the ECS continues rearrangement and maturational processes during adolescence. Developmental binding studies 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 decreased to adult values (Rodriguez de Fonseca et al., 1993). As indicated above, during the

period of rapid CB_1 cannabinoid receptor development, numerous neurodevelopmental alterations take place; including myelination, synaptic pruning and the maturation of neurotransmitter systems such as the dopaminergic, serotonergic and glutamatergic systems with which the cannabinoid system establish functional relationships. The implication of the ECS in brain developmental processes may explain the negative effects of adolescence cannabinoids consumption on emotional and cognitive function (Sections 3.2 and 3.3) as well as cognitive deficits observed in children born from women who used marijuana during pregnancy (Mereu et al., 2003).

In their developmental study quoted above, Rodriguez de Fonseca et al. (1993) 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 (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., 2007). 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 to male animals (Mateos et al., in press). 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.

3.2. 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 the last years (6.7%), marijuana has been the illicit drug with the highest rate of dependence or abuse in the last 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 agonists during the peri-adolescent period causes persistent behavioral 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 arises as a phase of development particularly vulnerable to at least some of the adverse effects of exposure to cannabinoid compounds. A 21-day treatment with CP 55,940 in 30-day-old rats resulted in a lasting impairment of working memory (O'Shea et al., 2004). Interestingly, these later behavioral changes were observed in adolescent but not adult drug-treated rats. In another study, chronic pubertal treatment with another cannabinoid agonist, WIN 55,212-2, resulted in impaired memory in adulthood (Schneider and Koch, 2003). In line with the study by O'Shea et al. (2004), these authors also showed that the chronic treatment with WIN 55,212-2 during adulthood did not lead to behavioral changes (Schneider and Koch, 2003). 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 behavior and self-grooming. Furthermore, acute administration of WIN 55,212-2 induced more severe effects on behavioral performance in pubertal than in adult rats (Schneider et al., 2008). Exposure of male rats to chronic delta-9-tetrahydrocannabinol (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 (O'Shea et al., 2006). 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., in press). Our results also indicated that, in the object location task, only the females showed a significantly impaired performance in response to adolescent cannabinoid exposure (postnatal days 28–43), suggesting that diverse aspects of memory function may be differentially affected in each gender (Mateos et al., in press). Rubino et al. (2009) showed that a sub-chronic treatment with THC from 35 to 45 postnatal days, resulted, in the adulthood (PND 75) in a worse performance in the radial maze, though no alteration was found in their aversive memory (passive avoidance). 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 the 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.

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 the last years while the rate did not change significantly for males (SAMHSA, 2008). In accordance with findings from rodents, human studies also suggest the existence of gender differences as regards cannabis-induced cognitive impairment in young people (Pope et al., 1997), though much more research is necessary evaluating sexual dimorphisms. It is very likely that long-term cognitive effects of adolescent cannabinoid exposure are related to less 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 the differential behavioral impact. A possible link between the

impaired memory observed specifically in males pre-exposed to cannabinoid in the novel object test and the increased functional activity of their CB_1 receptors (Mateos et al., in press) 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 behavioral 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 to 45 days of age). We used a battery of tests which provide complementary data about diverse aspects of the spontaneous behavior of the animals and their anxiety-related responses. In the holeboard test, CP 55,940-treated females showed a decreased general motor activity, and a significantly increased head-dipping duration (an exploratory parameter). In contrast, males treated with CP 55,940 in youth 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 (see Viveros et al., 2005a for review). As for other type of emotional responses, Rubino et al. (2008) demonstrated that THC chronic administration in adolescent rats induced subtle but lasting alterations in the emotional circuit ending in depressive-like behavior in adulthood and that this effect was observed in females but not in male rats (Rubino et al., 2008).

3.3. Vulnerabilities during the periadolescent period

Adolescence is a time of increasing incidence of several classes of psychiatric illnesses, including anxiety and mood disorders, psychosis, eating disorders, personality disorders and substance abuse. The pathophysiology of these disorders is being increasingly understood as arising from aberrations of maturational changes that normally occur in the adolescent brain (Giedd et al., 2008). This increased vulnerability to pathological alterations may be in part the consequence of an inadequate plastic brain remodeling during prepubertal maturation, puberty and adolescence (Walker et al., 2004). Large imaging studies have shown that brain changes associated with schizophrenia typically begin in adolescence when the brain undergoes the normal pruning sequence of myelination growth spurts and grey matter loss. It appears that a larger and more severe wave of grey matter loss occurs in the brains of adolescents developing schizophrenia, which eventually engulfs much of the cortex after a period of 5 years (Thompson et al., 2001) and it has been detected that the rates of brain volume reduction during adolescence are significantly higher for patients with childhood-onset schizophrenia than for healthy comparison subjects (Sporn et al., 2003). The marked sex differences in age of onset, prevalence and symptomatology for nearly every neuropsychiatric disorder may provide important clues as to their pathophysiology. The most obvious outward physical manifestations of puberty are caused by changing levels of hormones, but the relationship between hormones, brain and behavior is complex, reciprocal

and relatively poorly understood. Moreover, in humans, cultural and social-familiar factors may be crucial (Giedd et al., 2008).

Modeling the adolescent phase in animals is useful for the investigation of risk for addictive and other early-onset neuropsychiatric disorders. The increasing risk to develop 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 behavior. While any 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 construct adequate animal models that may be useful to unravel disease mechanisms and that may allow testing novel interventions (Adriani and Laviola, 2004; Giedd et al., 2008). In this section, we will focus on cannabinoid-induced neurpsychiatric disorders, with special emphasis in psychotic sympthomatology.

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. 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 the relationship between cannabis use and schizophrenia. A dysregulation of the endocannabinoid system may be implicated in the pathogenesis of schizophrenia. The peripubertal period appears to be critical for the development of cannabinoid CB1 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 endocannabinoid system 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 analyzing the association between adolescent cannabis use and the long-lasting development of psychotic symptoms. For example, chronic pubertal treatment with another cannabinoid agonist, WIN 55,212-2, resulted in impaired memory in adulthood as well as in a disrupted prepulse inhibition PPI of the acoustic startle response and lower breakpoints in a progressive ratio operant behavior 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. Interestingly, chronic WIN 55,212-2 treated rats not only showed a higher baseline Fos IR in the nucleus accumbens (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 behavioral alterations observed during adulthood, possibly affecting the adult organism's response to certain drugs of abuse (Wegener and Koch, 2009). As the vast majority of this kind of studies, this one was performed in male rats. In the next section we will see that in fact adolescent exposure to cannabinoid result 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. It would be very interesting to directly address possible sexual dimorphisms regarding increased risk to show schizophrenic-like symptoms in adolescent animals exposed to cannabinoids. In this point it is worth mentioning that a neonatal maternal deprivation stress that has been proposed as a model of psychotic-like symptoms (Ellenbroek et al., 1998) induced sex-dependent cellular and biochemical alterations, including effects on the endocannabinoid system, in the developing brain. From our work on this model, we propose that sex differences affecting at least certain psychiatric illnesses may have an origin in early development (Viveros et al., 2009).

3.4. 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). However, when we evaluated the effects of the same cannabinoid agonist on the anxiety-related responses (plus-maze) of 40-day-old male and female rats, the data indicated a different profile. We have performed a dose–response curve within a wide range of doses (0.1–100 mg/kg). The most intriguing 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). This is consistent with animal studies showing that nicotine may enhance the physiological, behavioral, and rewarding effects of THC (Valjent et al., 2002). We addressed the effects of a subchronic treatment with nicotine upon acute behavioral responses to the cannabinoid receptor agonist CP 55,940 in adolescent rats of both genders. With respect to anxiety-related responses in the plus-maze we found that, 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 might be a risk factor for the consumption of other drugs of abuse (Lynskey et al., 2003) though the alternative 'correlated liabilities model' proposes that cannabis use and other illicit drug use is influenced by correlated genetic and environmental factors (Agrawal et al., 2004). Also in this case, the use of animal models has been very useful to analyze possible neurobiological substrates for these interactions. Sex-specific patterns of consumption during all phases of drug addiction are well documented (see for review 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 functionality of μ-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, exposure to the cannabinoid agonist affected μ-opioid receptor binding and functionality in a sex-dependent manner in adult rats. Thus, adolescent CP 55,940 exposure also increased μ-opioid receptor levels in the subcallosal streak of pre-treated animals and decreased μ-opioid receptor functionality in the nucleus accumbens shell but again, only in males (Biscaia et al., 2008). According to our results, decreased μ-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 μ-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). In our above indicated study (Biscaia et al., 2008), although the effects of CP 55,940 were not so prominent in other brain regions, the cannabinoid agonist tended to produce more general sex-dependent changes in μ-opioid receptor binding in adult rats. Thus, an overall decrease was found in the cingulate cortex, CA2 and CA3 of the hippocampus, and in several thalamic nuclei of males exposed to CP 55,940, while an overall increase was observed in the same brain regions in females exposed to CP 55,940. The direction of sex differences regarding long lasting effects of adolescent cannabinoid exposure on self-administration of other drugs of abuse may depend of the specific nature of the drug. By using a similar (though not the same) 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) analyzed 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. Additionally, brain metabolic activity was analyzed by means of [^{18}F]-fluorodeoxyglucose positron emission tomography. During the acquisition phase, female CP 55,940-treated rats showed a higher rate of cocaine self-administration as compared to vehicle-treated females and males, whereas no differences were found between both male groups. This effect disappeared in the maintenance phase. Basal brain metabolic activity also changed in CP 55,940-treated females when compared to their vehicle-treated counterparts with no differences being found in the males. These alterations consisted of a hyperactivation of the frontal cortex and a hypoactivation of the amygdalo-enthorinal area (Higuera-Matas et al., 2008).

3.5. Organizational vs. activational effects of gonadal steroid hormones in the establishment and maintenance of sex differences in the biological actions of cannabinoids

As indicated in the previous sections, there are a large amount of reports describing sexual dimorphisms affecting the endocannabinoid system and cannabinoid-mediated physiological and pathphysiological processes. However, 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. Thus, in the studies showing that women are more vulnerable to the cannabinoid-induced memory impairment (Pope et al., 1997), as well as the orthostatic hypotension precipitated by the transition from the supine to the standing position (Mathew et al., 2003), it is difficult, if not impossible, to say anything about how estrogens, progesterone and androgens influence subject responsiveness to these cannabinoid effects. The same holds true for the comparatively greater subjective behavioral ratings of cannabinoid-induced euphoria, as well as the more potent cannabinoid-induced tachycardia observed following nicotine pretreatment, reported in men (Penetar et al., 2005). Even in many animal studies, where endocrine status can be more precisely controlled, the reported sex differences were based off of simple comparisons between gonadally intact males and females without regard to the hormonal profile at the time of endpoint determination. Thus, despite the fact that female rodents express higher aldehyde oxygenase activity (Watanabe et al., 1992) and more readily convert THC to the bioactive metabolite 11-hydroxy-THC (Narimatsu et al., 1991, 1992), and that male rats more robustly consume sweetened condensed milk in response to centrally administered cannabinoid CB_1 receptor agonists (Miller et al., 2004), we do not know how gonadal steroids establish these disparities during early development or how they maintain them during adulthood.

The ideal way to test for sex differences in a given cannabinoid effect is to perform direct comparisons between castrated male and female subjects. At the very least one should control for cyclical fluctuations in the gonadal steroid hormone levels of intact subjects by evaluating cannabinoid action at a particular stage of the estrous cycle and time of day. Thus, cannabinoid CB_1 receptor density in the medial basal hypothalamus, and expression in the anterior pituitary, varies over the course of the estrous cycle (Rodriguez de Fonseca et al., 1994) – with the lowest levels observed during estrus – and, in the case of the latter, is

higher in males than in females and is increased following ovariectomy in an estrogen-reversible manner (Gonzalez et al., 2000). This suggests that CB_1 receptor expression is sexually differentiated in a regionally specific way and subject to acute activational suppression by estrogen. There is also evidence for activational effects of gonadal steroids on the cannabinoid regulation of hypothalamic neuropeptide expression. For example, estrogen *per se* decreases the expression of preproenkephalin (PENK) in the hypothalamic paraventricular and ventromedial nuclei, and promotes THC-induced increases in expression (Corchero et al., 2002). It also facilitates enhanced POMC gene expression in the ARC (Corchero et al., 2001). On the other hand, while androgens have no effect on the THC-induced increases in PENK and POMC expression, they exert a permissive effect that enables THC to elevate corticotrophin-releasing hormone expression in the hypothalamic paraventricular nucleus (Corchero et al., 2001, 2002). The sex differences reported for the CB_1 receptor-mediated regulation of food intake and body temperature (more robust hyperphagia and hypothermia in males), as well as the changes in synaptic transmission occurring at POMC synapses (i.e., ~6× greater agonist potency to presynaptically inhibit GABA release in females, activation of the G protein-gated inwardly rectifying K^+ current in males but not females, augmentation of the A-type K^+ current (I_A) in females but not males), were ascertained through comparisons between orchidectomized male and ovariectomized female guinea pigs (Farhang et al., 2009). This indicates that *in utero* and/or neonatal exposure to androgens, which drives the development of the central nervous system in the guinea pig just as it does in the primate (Resko and Roselli, 1997), also plays an integral role in the establishment of sex differences in the cannabinoid regulation of energy homeostasis. In addition, estrogen has been shown to produce a rapid and sustained attenuation of the cannabinoid-induced hyperphagia and hypothermia, as well as the presynaptic inhibition of glutamate release and enhancement of I_A at POMC synapses, in ovariectomized female guinea pigs (Kellert et al., 2009; Nguyen and Wagner, 2006). This demonstrates that estrogen can act acutely to further diminish cannabinoid-induced alterations in energy balance. Although earlier reports of sex differences in the antinociceptive and locomotor actions of cannabinoids involved comparisons between gonadally intact rats and mice (Tseng and Craft, 2001; Wiley, 2003), Craft and Leitl (2008) showed that testosterone decreased the hypolocomotor but not antinociceptive effects in orchidectomized male rats, whereas estrogen accentuated the cannabinoid-induced antinociception in ovariectomized female rats but was without effect on locomotion or catalepsy (Craft and Leitl, 2008). Moreover, increases in paw-pressure antinociception were enhanced in estrus vs. diestrous females. This suggests that gonadal steroids exert activational effects on these cannabinoid-regulated processes—with androgens negatively modulating the cannabinoid-induced hypolocomotion and estrogens positively modulating the cannabinoid-induced antinociception. 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 that is reduced following ovariectomy (Fattore et al., 2007). Thus, while there is clearly a prevalence of sex/gender differences in the myriad of 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.

According to the original organizational–activational hypothesis of brain sexual differentiation, exposure to steroid hormones early in development masculinizes and defeminizes neural circuits (structural changes), programming behavioral 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 behaviors (activational effects) (Handa et al., 2008; Schwarz and McCarthy, 2008). Recent data has shed new insights into the mechanisms underlying sex differences. Thus, it has been proposed that the adolescent brain, undergoing remodeling, is organized a second time by gonadal steroid hormones secreted during puberty. This second wave of brain organization would build on and refines circuits that were sexually differentiated during early neural development. In fact, if steroid-dependent organization of behavior occurs during adolescence this prompts a reassessment of the developmental time-frame within which organizational effects are possible (Schulz et al., 2009). This idea opens new avenues for the investigation of mechanisms underlying gender differences in cannabinoid biology.

4. Concluding remarks

Adolescence is characterized by intense growth, reshaping and maturation of the grey and white matters in the human brain, with marked regional and sex differences. During this developmental phase, the nervous system shows a unique plasticity, and maturation and rearrangement of major neurotransmitter pathways and functions are still taking place (Adriani and Laviola, 2004; Crews et al., 2007; Spear, 2000). In general, there are important maturational changes in several brain areas important for cognition, reactivity to stress, emotion and motivation, such as the prefrontal cortex and limbic brain structures. Evidence obtained from both human and animal studies indicate that there are sex specific modifications in neurogenesis, neuronal death, dendritic complexity and synaptic connectivity during adolescence. The reorganization of brain structure during adolescence may reflect, at least in part, a process of refinement of neuronal networks, in adaptation to the new physiological conditions and are associated with the maturation of new specific behaviors. Natural teenage process of pruning may be accelerated or otherwise altered in schizophrenia, bipolar disorder, and other neurodevelopmental disorders. Therefore, adolescence could be considered as a “window of vulnerability” in relation to the onset of certain neuropsychiatric disorders, including an increased vulnerability to addiction (Adriani and Laviola, 2004; Crews et al., 2007; Spear, 2000; Witt, 2007). On the other hand, the high level of plasticity in the adolescent brain allows therapeutic interventions to compensate for the negative impact of early stressful experiences in life. Thus, exposure to an enriched environment during adolescence is able to compensate or reverse the effect of prenatal stress on cognition, play behavior, depressive behavior and emotionality in rats (Cui et al., 2006; Laviola et al., 2004; Morley-Fletcher et al., 2003). So, the establishment of the specific windows of intervention and the analysis of possible sexual dimorphism in this respect is a crucial matter.

The endocannabinoid system is crucially involved in brain development (Fernandez-Ruiz et al., 2004; Harkany et al., 2007) and appears to be an important component of the brain reward system (Gardner, 2005). Considering that the periadolescent period is critical for maturational processes of the endocannabinoid system, it is not surprising that the

developing brain is highly susceptible to cannabis exposure. Important sex differences have emerged in relation to the psychobiology of cannabinoids and there is an urgent need for further animal and human studies in this area. The emotional impact of drugs may be important both for the initiation and the maintenance of drug-taking behavior. Animal studies suggest that cannabinoids may interact with other drugs of abuse such as nicotine in the modulation of anxiety-related responses in a sex-dependent manner. Given the crucial role of the endocannabinoid system in the control of homeostasis, the findings about its sexual dimorphisms may help development of novel treatments for stress, depression, addiction, obesity, as well as HIV/AIDS- and cancer-related cachexia that are designed specifically for women and men.

Advances in adolescent brain research are leading to a better understanding of the growing adolescent brain, both in typical and atypical development. However, there is relatively scarce information about a possible differential vulnerability of the two sexes to diverse kinds of stressful events of pharmacological interventions and addiction-related processes. Earlier detection of atypical brain changes that may serve as markers for mental diseases, including dual pathology (comorbidity of mental illness and addiction) later in life, may lead to improved and targeted interventions. The findings reviewed in this article highlights the urgent need to consider gender/sex as a crucial factor in epidemiological, clinical and preclinical research.

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