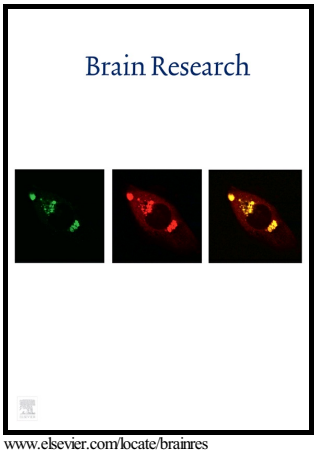

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PII: S0006-8993(16)30597-2
DOI: http://dx.doi.org/10.1016/j.brainres.2016.08.037
Reference: BRES45082

To appear in: *Brain Research*

Received date: 21 June 2016
Revised date: 23 August 2016
Accepted date: 25 August 2016

Cite this article as: Diana Dow-Edwards and Lindsay Silva, Endocannabinoids i brain plasticity: cortical maturation, HPA axis function and behavior, *Brai Research,* http://dx.doi.org/10.1016/j.brainres.2016.08.037

Brain Research Special Issue: Adolescence as a critical period for developmental plasticity

Endocannabinoids in brain plasticity: cortical maturation, HPA axis function and behavior

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Abstract

Marijuana use during adolescence has reached virtually every strata of society. The general population has the perception that marijuana use is safe for mature people and therefore is also safe for developing adolescents. However, both clinical and preclinical research shows that marijuana use, particularly prior to age 16, could have long-term effects on cognition, anxiety and stress-related behaviors, mood disorders and substance abuse. These effects derive from the role of the endocannabinoid system, the endogenous cannabinoid system, in the development of cortex, amygdala, hippocampus and hypothalamus during adolescence. Endocannabinoids are necessary for normal neuronal excitation and inhibition through actions at glutamate and GABA terminals. Synaptic pruning at excitatory synapses and sparing of inhibitory synapses likely results in changes in the balance of excitation/inhibition in individual neurons and within networks; processes which are necessary for normal cortical development. The interaction between prefrontal cortex (PFC), amygdala and hippocampus is responsible for emotional memory, anxiety-related behaviors and drug abuse and all utilize the endogenous cannabinoid system to maintain homeostasis. Also, endocannabinoids are required for fast and slow feedback in the normal stress response, processes which mature during adolescence. Therefore, exogenous cannabinoids, such as marijuana, have the potential to alter the course of development of each of these major systems (limbic, hypothalamic-pituitary-adrenal (HPA) axis and neocortex) if used during the critical period of brain development, adolescence.

Key words

Pruning and endocannabinoids
HPA axis development
PFC prefrontal cortex
Stress and CB receptors

Stress and CB responses
Sex differences

Abbreviations

2-AG	2-arachidonoylglycerol
ACTH	adrenal corticotropic hormone
AEA	*N*-arachidonoylethaonlamine/anandemide
BLA	basolateral amygdala
CB	cannabinoid
CB1R	cannabinoid receptors Type 1
CP	CP 55,940
CRH	corticotrophin releasing hormone
GABA	gamma amino butyric acid
HPA	hypothalamic pituitary adrenal
LTD	long-term depression
LTP	long-term potentiation
MS	maternal separation
NMDA	N-methyl-D-aspartate
PND	postnatal day
PVN	paraventricular nucleus
THC	tetrahydrocannabinol

Introduction

Adolescence is a time of experience-dependent pruning of the neuropil and thus a period which is highly vulnerable to environmental insults and influences. Much work has been done on the development of the brain during adolescence in recent years and a prominent role for endocannabinoids (the endogenous ligands for the cannabinoid receptor) in cortical—especially prefrontal cortical—development and the development of emotional regulation and stress responsivity has been described. This paper will present an overview of the endocannabinoid system, its role in plasticity, emotional regulation and stress responses. Then we will discuss how early stress alters endocannabinoid tone which in turn alters the behavioral and biochemical responses to exogenous cannabinoids (CB). Not surprisingly, there are robust differences between males and females in most of these responses and these differences will be discussed as well.

Retrograde signaling by endocannabinoids

The endocannabinoid system is the major retrograde signaling system in the brain, as activation of presynaptically located cannabinoid 1 receptors (CB1R) influences neurotransmitter release from presynaptic cells. CB1R are distributed throughout the brain being the most abundant G-protein-coupled receptor in the brain. However, they are expressed heterogeneously, with the highest concentration in cortex, hippocampus, amygdala, and striatum (Herkenham et al., 1991). The two primary endocannabinoids, N-arachidonoylethanolamide (anandamide/AEA) and 2-arachidonoylglycerol (2-AG), are synthesized and released by activation of the post-synaptic cell and typically inhibit presynaptic neurotransmitter release. CB1R are commonly expressed in glutamatergic and GABAergic synapses where their activation results in

suppression of either the excitatory or inhibitory output, respectively (Alger, 2002). AEA is degraded by the enzyme fatty acid amide hydrolase and 2-AG is metabolized by monacylglyceride lipase (Rodriguez de Fonseca et al., 2005). The formation of the endocannabinoids in response to neurotransmitter binding on the postsynaptic cell along with their rapid degradation give the endocannabinoids a unique temporal and spatial specificity that is not achieved with exogenous cannabinoids.

Normal ontogeny of the endocannabinoid system

Endocannabinoids within each brain region and sex undergo specific patterns of development during adolescence (figure 1). In males, AEA exhibits a gradual and progressive increase in PFC (i.e. postnatal day (PND) 29–50) to adult levels while 2-AG is already maximal during early adolescence (PND 29). However, 2-AG decreases in mid-adolescence (PND 38) only to increase again in late adolescence (PND50; Ellgren et al., 2008). AEA and 2-AG levels in nucleus accumbens and striatum exhibit unique developmental trajectories (Ellgren et al. 2008). Looking at regional AEA levels in male Sprague-Dawleys, Lee et al (2013) reported that in PFC, hippocampus, amygdala and hypothalamus AEA levels increase from PND 25 to 35, then decrease to PND 45 only to increase again to adult levels. FAAH activity shows a reciprocal pattern overall (Lee et al, 2013). In the female, the AEA concentration in PFC increases from PND 46 to 60, but decreases from PND 60 to 75, with no alterations in 2-AG concentrations (Rubino et al., 2015). Most authors report that CB1R are maximal early in adolescence and then gradually decrease to adult levels in PFC (Ellgren et al., 2008, Heng et al., 2011), with declines in limbic/ associative regions happening gradually during adolescence whereas changes in sensorimotor regions are not exhibited until mid- to late-adolescence (Heng et al., 2011).

Plasticity and cortical development

Synaptic plasticity can be defined as a modification of the nervous system by experience. Associative or Hebbian plasticity is the result of coincident or discordant presynaptic and postsynaptic firing. That is, recent firing history of neurons can produce alterations in synaptic strength. Long-term potentiation (LTP) occurs when the synapse strengthens (with coincident firing) and long term depression (LTD) when the synapse weakens (with discordant firing). LTP was initially characterized in the hippocampus by Bliss and Lomo (1973) in CA1 of the hippocampus by high frequency stimulation of the perforant path. LTP and LTD have roles in both learning and development and this review will focus on development. Mark Bear and colleagues were responsible for the identification that both LTP and LTD were involved in the development of the ocular dominance columns in visual cortex (Bear, 2003). Through an elegant series of studies, the NMDA receptor was identified as the critical switch with changes in ratio of the NR2A/NR2B subunit expression following either the presence or absence of visual experience (Bear, 2003). Pruning occurs at glutamate synapses following discordant excitation of pre and post synaptic elements resulting in LTD. Therefore, correlated activity is necessary for synaptic survival (Selemon, 2013).

Many studies have shown that sensory input is necessary for proper cortical development. For example, trimming of the whiskers during early adolescence prevents the normal loss of dendritic spines in primary somatosensory cortex (Zuo et al., 2005). Pruning, or the normal loss of dendritic expansion, occurs predominantly at asymmetric synapses located on dendritic spines (Brenhouse and Andersen, 2011). These synapses are primarily glutamatergic excitatory synapses (Bourgeois and Rakic, 1993). Similar processes are suspected to occur in prefrontal cortex. Bossong & Niesink (2010) published a comprehensive review of molecular changes in prefrontal cortex during adolescence. They proposed that just as sensory input shapes sensory cortex, cognitive, social and emotional input shapes prefrontal cortex in an

orderly sequence (Bossong & Niesink, 2010). Afferent activity to cortex from subcortical regions such as amygdala, accumbens and hippocampus provides stimuli which result in either LTP (if synchronized) or LTD (if asynchronized) and subsequent strengthening (and retention) or weakening (and loss) of synapses (Bear, 2003; see review by Selemon, 2013). This loss of synapses or pruning is generally believed to result in a thinning of the cortex, a sign of maturation. Maturation not only involves a thinning of the gray matter, but also an increase in the extent and organization of the white matter as well as an increase in connectivity between cortical and subcortical areas (Giedd et al., 1999, Paus, 2005). These processes occur in prefrontal cortex throughout a protracted period of adolescence (Giedd et al., 1999).

Endocannabinoids and plasticity

The endocannabinoid networks operate in both phasic and tonic modes differentially affecting short-term and long-term plasticity throughout the brain (reviewed in Katona and Freund, 2012). Phasic endocannabinoid signaling mediates short term synaptic depression of excitation and inhibition and involves both homosynaptic and heterosynaptic forms of plasticity. Also, endocannabinoids readjust synaptic gain in response to persistent changes in neural activity. Tonic endocannabinoid activity can reduce GABA release from axon terminals in the hippocampus through actions at the CB1R (Losonczy et al. 2004).

Exogenous cannabinoids have been found to alter cortical development of both somatosensory and the prefrontal cortex. Liu et al (2008) demonstrated in the juvenile mouse that blockade of the CBR in visual cortex prevents plasticity in layer II/III while leaving layer IV plasticity intact. This supports the highly selective role of the CB1R in plasticity underlying the development of the ocular dominance columns. Administration of WIN, the synthetic CB agonist, later during pre-adolescence modulates synaptic strength and plasticity in prefrontal cortex (PFC) glutamatergic synapses (Auclair et al, 2000). Lovelace et al. (2015) have demonstrated that

exogenous cannabinoids during adolescence produce abnormal LTD in prefrontal cortex (PFC) by disrupting two types of long term depression: LTD mediated by metabotropic glutamate receptors and LTD mediated by CB1R.

In fact, Rubino et al (2015) reported that adolescent THC disrupted the GluN2A/GluN2B balance throughout the post treatment period and resulted in an increase in PSD95 (postsynaptic marker at glutamate synapses) and a long-term decrease in spine density in layer II/III pyramidal neurons in PFC. The presence of exogenous cannabinoids during cortical development produces lasting alterations in endocannabinoid signaling, endocannabinoid-mediated LTD and maturational changes in NMDA subunits in prefrontal cortex (Rubino et al., 2015, Renard et al., 2016).

Other endocannabinoid roles in cortex

Adolescence is a period during which synchronized neural network activity described as cortical oscillations matures (Uhlhaas et al., 2009) and the anatomical and physiological function of neural networks develops (Giedd et al., 1999, Gogtay et al., 2004, Uhlhaas et al., 2010). Raver and Keller (2014) demonstrated that early adolescent CB exposures permanently alter cortical oscillations with selective sensitivity of the PFC (compared to the somatomotor cortex). Cass et al (2014) reported that early and mid-adolescent WIN exposure resulted in a frequency-dependent PFC disinhibition due to impaired PFC GABAergic transmission in the adult. When WIN was administered after PND 50, this disinhibition was not seen supporting the increased sensitivity of the early rather than the late adolescent period. An orderly ontogeny of all these systems is critical for the development of normal cortical oscillations which are the underpinnings of all cognitive and sensory processing (Buzsaki and Draguhn, 2004).

Endocannabinoids have also been implicated in homeostatic regulation of network activity patterns (Freund et al., 2003, Gerdeman and Lovinger, 2003, Piomelli, 2003, Raver et al., 2013,

Sales-Carbonell et al., 2013). The distribution of endocannabinoid synthesizing and metabolizing enzymes, as well as that of the CB1R itself, suggests that endocannabinoids function differently at specific synapses and microcircuits in brain. CB1R on GABA terminals are involved in THC-induced long-term memory deficits while those associated with glutamate neurons are involved in seizures (reviewed in Katona and Freund, 2012). Similarly, feeding and energy balance are differentially controlled by CB1R; those on striatal GABAergic neurons decrease food intake whereas CB1R on forebrain glutamatergic neurons increase food intake. Therefore, endocannabinoid signaling at distinct synapses regulates specific behaviors which are mediated by distinct neuronal circuits (reviewed in Katona and Freund, 2012). Exogenous cannabinoids such as THC administered during adolescence can alter functional networks in the prefrontal cortex, disrupt LTP and LTD and promote abnormal development of many abilities including cognition, emotional regulation and behavioral competency.

Exogenous cannabinoids in cortical development

Exogenous CBs such as THC or the synthetic CB1R agonists appear to arrest maturation of the cortex. Many structural imaging studies support this although the specific regions affected vary from study to study (see review by Batalla et al, 2013). Since maturation in the prefrontal cortex occurs relatively late in young adulthood and is highly sensitive to modification in endocannabinoid function, this region is uniquely vulnerable to disruption by exogenous CBs. Smoking marijuana during adolescence, particularly prior to age 16, is widely associated with impaired cognition, increased risk for psychiatric disease such as schizophrenia and depression as well as dysregulated executive function and increased propensity for substance abuse (D'Souza et al., 2005, Arseneault et al., 2004, Solowij et al., 2011, Hurd et al., 2014). Since the endocannabinoid system undergoes extensive reorganization during adolescence (Ellgren et al., 2008) and maturation of the PFC networks rely on proper endocannabinoid function (Renard

et al., 2016, Rubino et al, 2015), disruption of normal PFC ontogeny by exogenous cannabinoids is believed to be the primary mechanism by which marijuana impairs cognition and executive function, increases the risk for psychosis, and increases the propensity for substance abuse (Arseneault et al., 2004)

In addition, endocannabinoid signaling within the PFC regulates stress responses and emotional behaviors (McLaughlin et al., 2014). Endocannabinoid signaling is critical to both activating and terminating the hypothalamic-pituitary-adrenal response to stress (Hill et al., 2010). Endocannabinoids are also involved in orchestrating the cognitive and emotional responses to stress through actions on the amygdala, hippocampus, and hypothalamus in addition to the prefrontal cortex (McLaughlin et al., 2014).

Development of the stress response and anxiety in adolescence

Stress responsivity and anxiety are regulated by corticolimbic structures including the amygdala, hippocampus and PFC (Romeo and McEwen, 2006). The ventromedial PFC is important for an individual's ability to shift from fear expression to fear suppression (Milad and Quirk, 2002, Santini et al., 2004, Milad et al., 2007). A deficiency in ventromedial PFC input to the amygdala is likely to result in a reduction of inhibitory tone within the latter structure, which could ultimately lead to the over expression of fear responses and development of an anxiety disorder. The amygdala and hippocampus have dense reciprocal connections. The hippocampus plays a role in the acquisition and storage of contextual fear memory. It provides information about the safety or threat of an environment based on contextual representations formed by previous experience (Fanselow and Dong, 2010, Orsini and Maren, 2012).

The HPA axis also matures during adolescence. Basal ACTH and corticosterone levels are relatively stable during puberty and adulthood and the response to an acute stressor (e.g.

restraint stress) is comparable between pre-pubertal (PND 28) and adult rats in terms of increases in ACTH and corticosterone (Romeo et al., 2004a, Romeo et al., 2004b, Pignatelli et al., 2006) . However, pre-pubertal male and female rats exhibit a prolonged, stress-induced corticosterone response when compared to adult rats (Romeo et al., 2004a, Romeo et al., 2004b). In adults, habituation of the corticosterone response occurs following repetitive exposure to the same stressor (e.g., Girotti et al., 2006), but pre-pubertal male rats exposed to repeated stressors do not show this same blunting of the corticosterone response. Actually, pre-pubertal males have higher peak ACTH and corticosterone levels and a more rapid normalization to baseline following repeated stressors (Romeo et al., 2006, Doremus-Fitzwater et al., 2009). Pubertal female rats, on the other hand, do not show this increase in corticosterone response (Doremus-Fitzwater et al., 2009). These changes in hypothalamic pituitary adrenal (HPA) responsivity have been tied to changes within the PVN. Its structural appearance does not change across adolescence (Romeo et al., 2007) while its responsivity transiently increases (Romeo et al., 2006). Hypothetically, the negative feedback by corticosterone which is mediated by endocannabinoids is undergoing maturation.

Role of endocannabinoids in development of the stress response

The endocannabinoid system is a necessary component of the glucocorticoid-mediated negative feedback loop which regulates the hypothalamic-pituitary-adrenal axis (Hill and Tasker, 2012). Glucocorticoids directly increase PVN content of AEA and 2-AG and rapidly suppress glutamate release from pre-synaptic CRH neurons in PVN providing rapid glucocorticoid-mediated negative feedback on the HPA. In addition to direct actions on the PVN, basal HPA tone is regulated by endocannabinoids since disruption (by a CB blocker) increases HPA axis output likely through extra-hypothalamic sites (Hill and Tasker, 2012). Stress decreases tissue content of AEA in BL amygdala, a region responsible for regulating basal HPA tone. This, in turn, activates BLA to inhibit inhibitory input to the PVN. Endocannabinoids are also involved in

glucocorticoid-mediated negative feedback through actions at prefrontal cortex, hippocampus and amygdala. This feedback is functional already at PND8 in rats and it undergoes maturation throughout adolescence (Doremus-Fitzwater et al., 2009, Buwembo et al., 2013). Endocannabinoids are also involved in regulating the HPA axis in response to both acute and repeated stress (Hill et al., 2010). Adolescent CB administration alters corticosterone responses in adulthood in sex-specific fashion with greater increases in peak corticosterone levels in treated males than females (Lee et al., 2014). That is, exogenous cannabinoids administered during PND 35 to 46 increase the corticosterone response to stress in adult males, but not females, hypothetically due to impaired feedback mediated by endocannabinoids.

Endocannabinoid signaling in corticolimbic structures such as the prefrontal cortex (PFC), amygdala and hippocampus plays a critical role in regulating emotional behavior such as anxiety and stress responses in the adult (Rubino and Parolaro, 2008, Campolongo et al., 2011, Lee and Gorzalka, 2012, Morena et al., 2016). Since the endocannabinoid system in these structures is undergoing maturational changes during adolescence and perturbation of this maturation with exogenous cannabinoids produces long-term alterations in stress responses and anxiety related behavior, an important role of the endocannabinoid system in the maturation of anxiety and stress related behaviors is suggested (Lee et al., 2016). Therefore, the presence of exogenous CBs during the maturation of the stress-response system produces a protracted dysregulation of stress responsivity which has been linked to dysregulation of mood states such as those related to depression, anxiety and substance use disorder (Andersen and Teicher, 2009, McLaughlin and Gobbi, 2012, McCormick and Green, 2013).

Early stress alters the endocannabinoid system

Maternal separation has been used as a stressor in rodent studies for many years. Although various procedures have been utilized (e.g., Meaney et al., 2007), maternal separation (MS) for 24 hr beginning on postnatal day (PND) 9 is a standard method of inducing stress to the rat pups. Ellenbroek et al. (1998) first described the procedure. Viveros et al. (2009) characterized the neuroendocrine, endocannabinoid and behavioral effects of PND 9 MS which differ depending on the sex of the animal. At PND 13 in males, hippocampal AEA levels were unchanged by MS but 2-AG levels were increased. CB1R were reduced in MS males and CB2 receptors were increased in both males and females following MS. Corticosterone and ACTH levels were initially increased at PND13 and then in adulthood, normalized in males but remained increased in females. Others have reported similar effects of early MS on the endogenous cannabinoid system (e.g., Lopez-Gallardo et al., 2012) with reductions in CB1R being reversed by exogenous cannabinoid administration in adulthood. However, others have reported that MS increases CB1R in frontal cortex (FrCx), and amygdala in males and increases CB1R only in hippocampus (with decreases in FrCx) in females when measured at PND 46 (Marco et al., 2014). Together these studies in Wistars suggest that MS initially (at PND 13) reduces CB1R (especially in males), then increases CB1R in frontal cortex and amygdala in males at PND 46 and then decreases CB1R in males in adulthood (Lopez-Gallardo et al., 2012). Interestingly, while most of the studies showing effects of MS on the endogenous CB system utilized offspring from females mated “in house”, Zamberletti et al. (2012) studied offspring of Sprague-Dawley rats shipped during pregnancy and found no overall effect of MS on CB1R. Shipping during pregnancy is most certainly a stressor (Stewart & Kolb, 1988, Bock et al., 2015) and we have shown that stress during pregnancy has its own effects on the endocannabinoid system which vary by the sex of the animal (Dow-Edwards et al., in revision). In fact, both prenatal stress and postnatal stress reduce CB1R in males and increase CB1R in females in most regions studied in

the mid adolescent brain (Dow-Edwards et al., in revision). For this study, we compared adolescent offspring of non-treated (NT) dams to offspring born of dams that received prenatal gastric intubations (a stressor) or alternatively with those born at the vendor, shipped at PND 21 and raised in a collaborators lab (we will refer to these as vendor-derived, VD). Prenatal stress (intubations) decreases CB1R in all regions assessed in males and increases CB1R only in striatum of females. Comparing the non-treated group with the VD group, we found in prefrontal cortex, striatum, hippocampus and amygdala, that being bred in a commercial facility and shipped at PND21 increased CB1R in females while in males, only the PFC and striatum showed substantial decreases in CB1R (figure 2). Therefore, prenatal/early postnatal stress histories alter basal CB1R in key limbic regions and these changes are sex-specific in that males show decreases and females show increases. Since the responses to exogenous cannabinoids are mediated by the CBR, these findings suggest that the early stress history sets the tone of the endocannabinoid system which would be expected to alter the behavioral responses to exogenous cannabinoids throughout life.

Sex differences in the endocannabinoid system and the behavioral effects of exogenous cannabinoids have been known for many years (see review by Fattore & Fratta, 2010). Since some behavioral effects of exogenous cannabinoids are more prominent in males while others are more prominent in females, sensitivity of the underlying neuronal circuits to cannabinoids is likely to differ between the sexes. Within females, most behavioral studies looking at estrous cycle-dependent effects find that exogenous cannabinoids produce greater effects during estrus when blood levels of estrogens are high compared to diestrus (e.g., Craft & Leitil, 2008). However, studies of CB1R across different phases of the cycle show variation in expression. CB1R in mediobasal hypothalamus is higher in diestrus compared to estrus while other brain regions do not show cycle-dependent alterations except in limbic forebrain where affinity

of the receptor was higher in diestrus (Rodriguez de Fonseca et al., 1994). Several groups report differences in receptor density between males and females with males generally showing greater receptor densities than females (Rodriguez de Fonseca et al., 1994, Reibe et al., 2010, Dow-Edwards et al., in revision). However, we have shown that the stress history of the rats can significantly alter the sexual dimorphism so it is challenging to compare binding densities across sexes in preclinical studies (Dow-Edwards et al., in revision).

Altered cannabinoid responses following maternal separation/early stress

Although Zamberletti et al. (2012) found that maternal separation had no effect on CB1R in adulthood in their study of offspring from shipped dams, administration of THC between PND 35-45 using an escalating dose paradigm, decreased CB1R in both males and females with females showing greater changes than males. There were no interactions between the MS and cannabinoid treatment in any of the 12 regions studied. Lopez-Gallardo et al. (2012) utilized maternal separation at PND 9 (Wistars mated “in house”) and then administered CP55,940, a synthetic cannabinoid agonist, at 0.4mg/kg ip or vehicle between PND 28-42 and examined the offspring at PND 80 for brain levels of receptors and found that MS decreased CB1R in males in several hippocampal subregions while CP treatment normalized these decreases. In females, MS reduced CB1R and the CP treatment normalized CB1R in CA1 stratum oriens but no other regions were affected (Lopez-Gallardo et al., 2012). Also, Wiley and Evans (2009) reported that shipping at weaning sensitized the responses to THC across a range of doses differentially in male and female rats. Therefore, there are clear sex differences in response to both early stress and cannabinoid treatments during adolescence and the prenatal stress history of the dam appears to contribute to the disparate results reported in many studies.

In order to directly assess the effects of early life shipping stress on adolescent THC responses, we administered 3mg/kg/day THC to adolescent (PND 29-38) male and female Sprague-Dawley rats that had either been born in our vivarium or bred in a commercial supplier and shipped on PND 14 and then raised in our vivarium along with the others (see Silva et al, 2016). Our results of behavioral tests conducted in mid-adolescence suggested that shipping interacts with adolescent THC exposure, and that these effects were sexually dimorphic. Adolescent (prepubertal) males were more susceptible to the anti-depressant-like effects of THC when they had been shipped from the supplier compared to those born in the vivarium, as shown by increased latency to adopt an immobile posture/passive coping mechanism in the forced swim test (FST) (figure 3). Also, pubertal females shipped from the supplier showed a stronger acoustic startle response following adolescent THC exposure than those born in the vivarium (figure 4), suggesting that these females may be hyper-reactive or hyper-vigilant, which could indicate increased anxious-like behavior. Also, these females showed increased CBR in central amygdala compared to controls and males which suggests a dysregulation in the emotional response system as well as the HPA axis (see full description of the study in Silva et al., 2016). As mentioned above, Wiley and Evans (2009) reported that shipping from a vendor on PND21 increased sensitivity to THC in males for certain behaviors and increased sensitivity to THC in females for other behaviors. All together, results suggest that early stress in the form of maternal separation or early shipping alters the development of the endocannabinoid system and results in long-term alterations in endocannabinoid markers and cannabinoid responsivity. Since early stress is associated with a range of neuropsychiatric disorders, it is possible that dysregulation in the endocannabinoid system may be involved in the etiology of these disorders.

Cannabis use in human adolescents

A complete review of the clinical effects of cannabis use during adolescence is beyond the scope of this review and several excellent reviews have already focused on this topic (e.g., Chadwick et al. 2013, Lubman et al, 2015, Leweke & Koethe, 2008). Several prospective longitudinal studies have demonstrated that marijuana smoking during the early teenage years is associated with drug addiction and major depressive disorder, as well as with psychosis (D'Souza et al., 2005, Arseneault et al., 2004, Solowij et al., 2011, Hurd et al., 2014). However, causality is difficult to demonstrate in human clinical studies and individuals with latent psychiatric disorders may be more likely to try cannabis than those without. Animal studies clearly demonstrate that significant early stressors promote altered endocannabinoid function and neuropsychiatric phenotypes. Cannabinoid exposure further compounds this altered function placing the adult at risk for a range of neuropsychiatric disorders including drug addiction.

Conclusions

The adolescent brain is undergoing extensive maturation and these processes are intimately associated with the maturation of the endocannabinoid system. Cortical development, which involves pruning, relies on normal endocannabinoid function as does the maturation of cortical network oscillations. Prefrontal cortex, the last cortical area to mature, is essential for multiple cognitive functions including executive function, managing stress responses, anxiety and mental health. Exogenous cannabinoids have been shown to alter the structure and function of the PFC. Stress responsivity develops during adolescence and endocannabinoids are essential for normal stress responses. Early stressors alter the endocannabinoid system (and the behavioral responses to exogenous cannabinoids) a factor which undoubtedly contributes to some of the disparate results published in the literature on the effects of exogenous cannabinoids on adolescent behavior and neurochemistry. However, use of exogenous cannabinoids

such as marijuana during adolescence can have significant, long lasting detrimental effects on cognition, anxiety, emotional regulation and stress responses.

One drawback of the animal studies is the use of routes of administration which have limited clinical relevance. It is well known that inhalation of substances produces rapid increases in brain concentrations and has very different effects on reward circuits and brain chemistry compared to other routes such as oral or even intraperitoneal injection. Researchers involved in nicotine/cigarette research have developed smoking machines for inhalational delivery and CB researchers should consider similar devices for drug delivery. In addition, although the THC content of marijuana has reached an all time high, natural cannabis contains many compounds (such as cannabadiol) some of which are antagonistic to THC. Consideration should be given to the possible effects of these other CB constituents in adolescent brain.

While a substantial body of work has already been published on the role of the endocannabinoids in the development of normal adolescent brain and behavior, the field still needs well-controlled longitudinal preclinical studies in addition to well-controlled, prospective, longitudinal clinical studies to establish a causal role of exogenous CBs in the development of altered cognition, emotional regulation, substance abuse and other psychopathology.

Acknowledgements:

The authors wish to acknowledge Fulbright Canada and the Palix Foundation for support during the writing of this manuscript. We also wish to thank Matt Hill, PhD for helpful comments made during the preparation of the paper.

Figures

Figure 1—Relative changes in HPA axis, endocannabinoid system, and cannabinoid receptors during postnatal development in the rat.

Figure 2 CB1 receptors as assessed by ^{3}H CP55,940 binding in prefrontal cortex, striatum, amygdala and hippocampus. Each region shows a significant sex by treatment (vendor derived or nontreated, NT) interaction ($P<0.05$, ANOVA). Post hoc analysis shows that receptors are significantly reduced in prefrontal cortex and striatum in vendor derived, shipped (VD) males compared to vivarium-reared non-treated (NT) males and increased in VD females compared to NT females . In amygdala and hippocampus, only VD females showed increases in CB1R. mean+sem (* $p<0.05$) (see full description of study in Dow-Edwards et al., in revision)

Figure 3. Average latency to immobility in the forced swim test in male and female rats treated with THC (3mg/kg) or vehicle during PND 29-38. Two days following the last dose, rats were tested in the forced swim test. Only those male rats shipped from the supplier on PND 14 and treated with THC showed an antidepressant phenotype. * indicates significantly ($p<0.05$) increased latency to immobility than shipped vehicle treated males (data derived from Silva et al, 2016).

Figure 4. Average initial (trial 1) startle response amplitude in male and female rats treated with THC (3mg/kg) or vehicle during PND 29-38. Ten days following the last dose, rats were tested in the acoustic startle paradigm. Only those female rats shipped from the supplier on PND 14 and treated with THC showed this enhanced initial startle response which could indicate a hyper-reactive/hypervigilant state. * indicates significantly ($p<0.05$) greater initial startle amplitude than vivarium reared THC treated females (data derived from Silva et al, 2016).

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Highlights

- Endocannabinoids are involved in adolescent brain maturation
- Endocannabinoids regulate plasticity through GABA and glutamate mechanisms
- Exogenous cannabinoids alter the development of cortex
- Exogenous cannabinoids may be associated with abnormal cognition, emotional regulation and substance abuse

Figure1

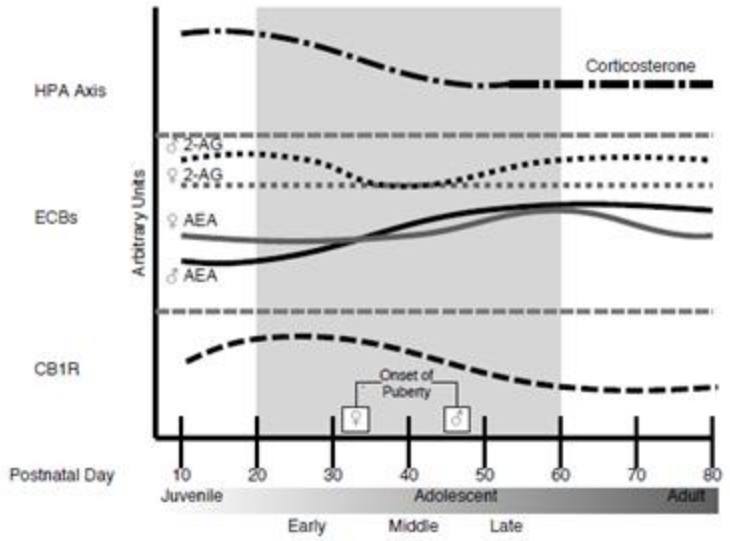

Figure 2

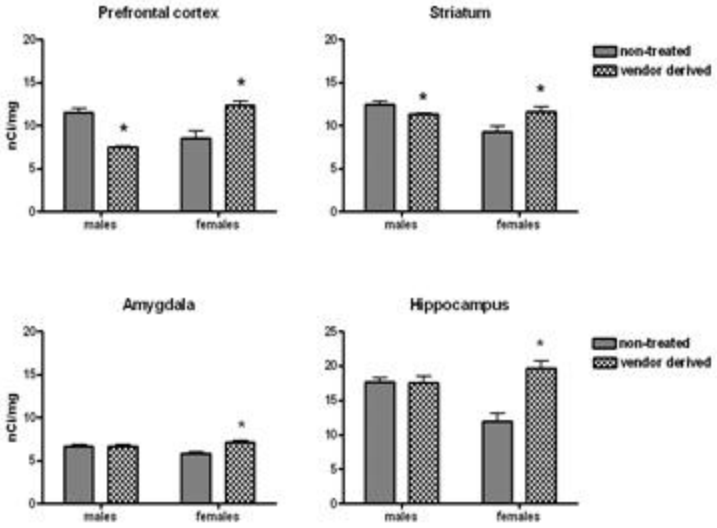

Figure 3

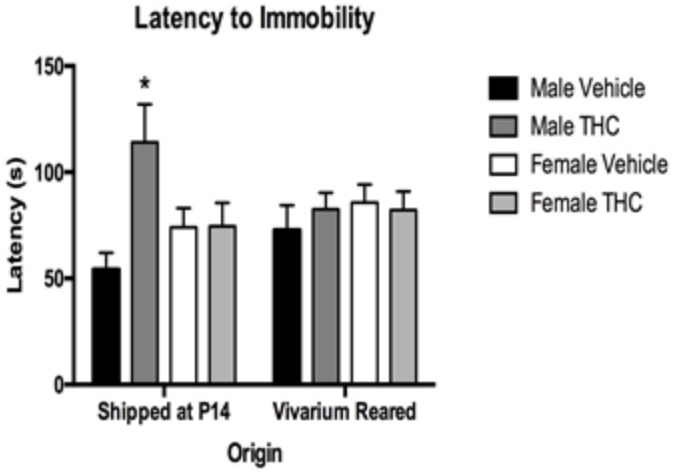

Figure4