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NEW VISTAS ON CANNABIS USE DISORDER

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Abstract

Cannabis sativa preparations are the most consumed illicit drugs for recreational purposes worldwide, and the number of people seeking treatment for cannabis use disorder has dramatically increased in the last decades. Due to the recent decriminalization or legalization of cannabis use in the Western Countries, we may predict that the number of people suffering from cannabis use disorder will increase. Despite the increasing number of cannabis studies over the past two decades, we have gaps of scientific knowledge pertaining to the neurobiological consequences of long-term cannabis use. Moreover, no specific treatments for cannabis use disorders are currently available.

In this review, we explore new research that may help fill these gaps. We discuss and provide a solution to the experimental limitation of a lack of rodent models of THC self-administration, and the importance this model can play in understanding the neurobiology of relapse and in providing a biological rationale for potential therapeutic targets. We also focus our attention on glial cells, commenting on recent preclinical evidence suggesting that alterations in microglia and astrocytes might contribute to the detrimental effects associated with cannabis abuse. Finally, due to the worrisome prevalence rates of cannabis use during pregnancy, we highlight the associations between cannabis use disorders during pregnancy and congenital disorders, describing the possible neuronal basis of vulnerability at molecular and circuit level.

Keywords

cannabis use disorder; THC self-administration; glia cells; perinatal cannabis; reward

1. Introduction

Social debate on mental health consequences of cannabis use has intensified in the last years due to the high rates of recreational cannabis use and the changing legal status of cannabis in

several Western countries. As a consequence of this higher use, demand for therapeutic treatments for cannabis use disorders (CUD) has increased worldwide since 2003 (World Drug Report 2016). CUD is associated with a broad range of health-related problems, such as cognitive decline, respiratory and cardiovascular diseases, psychiatric symptoms, and risk of addiction or substance use disorders (SUD; Volkow et al. 2014). Despite the high prevalence of CUD and the increasing number of cannabis users seeking treatment (World Drug Report 2016), to date no specific pharmacotherapy has been approved by any national regulatory authority. Current therapies are aimed at alleviating symptoms of cannabis withdrawal and include compounds that directly affect endogenous cannabinoid signaling or drugs efficacious in treating psychiatric conditions associated with other drugs of abuse (Gorelick, 2016). However, none of these medications has been proven broadly and consistently effective. Thus, an in-depth understanding of the neurobiological underpinnings of CUD is needed to provide potential new therapeutic targets.

In this review, we address some interesting findings that have been recently described regarding CUD. These novel insights into the neurobiological basis of CUD may help pave the way for new therapeutic approaches.

2. Why do we still lack a rodent model of cannabis self-administration?

Although cannabis is the most widely used illegal drug in the world (Borgelt et al. 2013), there is relatively little understanding of the neurobiological consequences of long-term cannabis use. The primary reason for our poor knowledge of CUD is the lack of experimental paradigms that model cardinal characteristics of addiction. Specifically, it has been difficult to establish a model of cannabis use in rodents that involves self-administration and drug seeking initiated by cues or contexts associated with cannabis delivery. Noncontingent (experimenter delivered) administration of the psychoactive ingredient of cannabis, Δ^9 -tetrahydrocannabinol (THC), is the currently used model, and provides understanding of the acute pharmacology of the drug and the neurobiological adaptations to repeated drug use. However, key to understanding relapse in particular is the integration between drug pharmacology and environmental or interoceptive stimuli that become associated with drug delivery (Shaham et al. 2003; Spencer et al. 2016). Thus, the lack of ability to produce learned associations with cannabis delivery in available animal models severely limits their utility in understanding the neurobiology of voluntary relapse to cannabis use. Even more critical, the lack of a model of voluntary use and highly motivated drug seeking limits the ability to use animal models in developing pharmacotherapies that might limit the motivation to relapse to cannabis use. The rodent model of cue- or context-induced relapse has proven successful in identifying novel biological targets for possibly treating other addictive drugs (Brown et al. 2013). Accordingly, the inability to model the neurobiology of relapse and to develop treatments for relapse is likely to be an expanding deficit as cannabis becomes decriminalized, legalized or medically legalized throughout the Western Countries.

2.1. Problems modeling cannabis self-administration and relapse

The majority of drugs abused by humans are also self-administered by rodents, lending strong face validity to this model. The standard model of drug self-administration varies in terms of time and dose of self-administration (Zernig et al. 2007). The period of self-administration ranges from weeks to months with a goal of either establishing stable intake in short 1–2 hr daily sessions or establishing escalated intake in extended 4–8 hr daily sessions of self-administration. To study relapse, the self-administration sessions are typically conducted daily in the same environment to create a contextual association. Also, many studies incorporate a Pavlovian discrete cue(s) that is associated with drug delivery. Irrespective of the precise self-administration protocol, relapse is evaluated after a period of withdrawal. The withdrawal period varies from 24 hrs to many weeks and is either a period of forced abstinence, or a period of daily exposure to the drug-paired context in order to “extinguish” the association the animal makes between drug and context (Shaham et al. 2003). Context extinction training is used to isolate the discrete drug associated cue as a trigger for reinstating drug-seeking. In the forced abstinence model, the animal is simply placed into the drug-paired context to initiate drug-seeking, although discrete cues may also be present. Initiating drug-seeking without drug access by either a drug-paired context or discrete cue is considered a model of high face validity since both types of stimuli can elicit craving and highly motivated drug-seeking in humans. Perhaps more importantly, these models of relapse may have predictive validity since compounds that successfully suppress relapse in these animal models are also successful at suppressing craving in clinical trials (Shaham et al. 2003; Spencer et al. 2016). Indeed, understanding the neurobiology underpinning this model of relapse for some drugs has provided rationales for introducing new compounds into clinical trials, specifically N-acetylcysteine for treating cocaine craving (Kalivas and Volkow, 2011; Brown et al. 2013).

The models outlined above have been successfully applied to most drugs that are addictive in humans, in particular amphetamine-like psychostimulants such as cocaine, opioids such as heroin, nicotine and alcohol. For the first three drug classes intravenous drug delivery is by far the most common route of administration, while for alcohol oral drug delivery is most common. The lack of a rodent model of THC self-administration and drug-seeking arises largely from four facts that taken together distinguish cannabis from other addictive drugs.

- a. Cannabis self-administration delivery systems are more difficult to establish than for most other addictive drugs. Human cannabis use is via inhalation or ingestion. Rodent models of voluntary drug inhalation are notoriously difficult to establish, as is revealed by the relatively few publications in the preclinical literature employing voluntary inhalation of tobacco smoke as a means of drug delivery (Harris et al. 2010). An alternative is intravenous (i.v.) drug self-administration, and i.v. self-administration of nicotine, the main psychoactive component of tobacco, has become the accepted model for studying tobacco addiction (Caille et al. 2012). Thus i.v. administration is the preferred model of self-administration for most addictive drugs but it has been difficult to establish for cannabis in rodents.

- b.** Cannabis, like nicotine, contains a number of psychoactive constituents, making the i.v. delivery of cannabis uncertain in terms of which constituent(s) to use. The primary psychoactive constituent of cannabis is THC, which is a partial agonist at both the cannabinoid CB1 and CB2 receptors (Pertwee, 2008). However, CB1 receptors are generally thought to be the primary site of action in brain contributing to the addictive properties of cannabis; although see (Zhang et al. 2014). With the exception of a series of publications in squirrel monkeys by Steve Goldberg's group (Tanda et al. 2000; Justinova et al. 2003, 2008), there is a paucity of literature on the successful de novo i.v. self-administration of THC in rodents. Rats previously trained to self-administer the CB1 receptor agonist WIN55,212-2 will subsequently self-administer THC (Lefever et al. 2014). In addition, THC is self-administered directly in the ventral tegmental area, or in the shell subcompartment of the nucleus accumbens (Zangen et al. 2006). It is possible that some of the negative consequences of systemic THC self-administration were circumvented by placing the THC directly onto the mesolimbic dopamine pathway, which is well-established to mediate both drug and natural reward learning (Cardinal and Everitt, 2004; Wise, 2004; Volkow et al. 2011).

The other primary constituent of cannabis is cannabidiol (CBD), which has relatively low affinity for CB1 and CB2 receptors and has numerous reported molecular targets in brain (Panlilio et al. 2015). Nonetheless, an abundant clinical and preclinical literature has developed showing that CBD may counteract, in part, the behavioral effects of THC, in particular its aversive and anxiety provoking effects (Russo and Guy, 2006; Iseger and Bossong, 2015). No efforts have been published attempting i.v. self-administration in rodents of either CBD or THC plus CBD (but see below). Another aspect of i.v. self-administration of either THC or CBD is that neither of these constituents is water soluble. Hence, they require ethanol or a detergent-based solvent such as Tween 80 to dissolve, thus adding to i.v. self-administration a potential vehicle confound.

- c.** Cannabis is a poor behavioral reinforcer. The poor rewarding properties of cannabis in rodents complicate the development of self-administration models. The reinforcing effects of THC have been investigated using a number of standard paradigms including drug discrimination, intracranial self-stimulation (ICSS), and conditioned place preference (CPP) with varied results. THC functions as a discriminative stimulus in rodents using a drug discrimination paradigm, and morphine potentiates the discriminative properties of THC (Solinas et al. 2004). Likewise, THC dose-dependently alters ICSS thresholds with a low dose (0.1 mg/kg) decreasing the threshold in line with a reward-facilitating function, while a higher dose (1 mg/kg) produces the opposite result (Katsidoni et al. 2013). The relatively poor rewarding properties of the constituents of cannabis are clearly seen in the classic CPP paradigm where THC is associated with a specific environment, and the animal is later given a choice to go into the drug-paired or the vehicle-paired environments. In contrast to its

apparent appeal in humans, most studies show no THC-induced place preference, although, as with ICSS, lower doses may produce CPP (Lepore et al. 1995; Valjent and Maldonado, 2000). In contrast, many studies reveal that THC elicits place aversion (spending less time in the THC associated versus neutral environment) or no CPP (Maldonado, 2002; Panlilio et al. 2015). Interestingly, in one study, CBD combined with THC blocked the place aversion induced by THC alone without eliciting place preference or aversion per se (Vann et al. 2008).

- d. THC long half-life and tissue depot. As discussed in detail below, THC accumulates in fatty tissue (Huestis, 2005). Having a long half-life may influence the reinforcement rates, as well as extinction responding and reinstatement of lever pressing. Thus, exploring different schedules of reinforcement and periodicity of drug self-administration sessions may influence responding for THC, and could be explored to further optimize the self-administration protocol outlined below and in figure 1.

2.2. Towards a model of self-administration and relapse

Although we outline numerous difficulties in creating a rodent model of cannabis self-administration, the literature described above contains two clues that we have recently incorporated in an effort to create a rat model of THC self-administration and relapse.

- a. Using a combination of THC and CBD to take advantage of the apparent capacity of CBD to decrease the aversive properties, which could unmask the relatively weak reinforcing property of THC; although, the studies with CPP did not show that THC became reinforcing in the presence of CBD (Vann et al. 2008).
- b. Pretreatment with noncontingent THC administration may create tolerance to its aversive effects. Indeed, among the few studies demonstrating CPP to THC, mice were pre-exposed to a priming injection of THC (Valjent and Maldonado, 2000). Employing the two principles described above, we endeavored to develop a relapse model of context- and cue-induced cannabis seeking that would permit analysis of the neurobiology underpinning cannabis relapse. We exposed rats to 5 daily treatments with noncontingent THC + CBD vapor (10:1 concentration ratio) prior to initiating 90 min daily sessions of intravenous THC + CBD using the same 10:1 dose ratio. Figure 1A shows combined data from two groups of rats trained to self-administer THC + CBD. Although lever pressing was low relative to drugs such as cocaine or heroin, the rats clearly distinguish the active from inactive lever by the end of training, and lever pressing was supported when rats were shifted to a lower dose of THC + CBD. A group of rats (n=8) underwent a period of forced abstinence for 10 days and when returned to the drug-paired context demonstrated a marked increase in active lever pressing relative to inactive lever pressing (figure 1B). This group then continued into extinction training to reduce active lever pressing in response to the drug-paired context, in preparation for a cue-induced reinstatement session. The remainder of the rats (n=6) began daily extinction training the day after the last self-administration session. The pooled extinction data for the two groups of rats is

shown in figure 1C, and on the last day of testing, all rats were reinstated by restoring cue presentation to active lever pressing. Importantly, the drug-paired light-tone compound cue was not presented in response to active lever pressing during extinction. Thus, when the light-tone cue was restored in extinguished rats, the cues reinstated active lever pressing relative to the inactive lever or extinction levels of active lever pressing (figure 1D).

3. Beyond neurons: impact of cannabis use on glial cells

Heavy cannabis use can produce severe morphological and neurophysiological abnormalities within brain structures high in CB1 receptors and subserving cognitive, executive and emotional processes (Lorenzetti et al. 2016). Importantly, these alterations appear to be greater when cannabis use takes place during critical developmental periods, such as adolescence. Indeed, heavy cannabis intake in adolescents can profoundly affect the brain refinement that takes place during this period and has been associated with marked alterations in behavior and brain functioning that persist until adulthood, thus increasing the risk for developing complex psychiatric disorders later in life (Rubino and Parolaro, 2016).

To date the vast majority of clinical and preclinical research investigating the molecular and cellular consequences of cannabis abuse on the brain has focused on cannabis-induced alterations in neuronal function and morphology, with particular attention to the GABAergic (Cortes-Briones et al. 2015; Radhakrishnan et al. 2015; Skosnik et al. 2014; Zamberletti et al. 2014) and glutamatergic (see for review Colizzi et al. 2016) systems. However, despite the central role played by neurons in mediating the effects associated with substance abuse, recent evidence suggests that alterations in glial cells, microglia and astrocyte in particular, contribute to the detrimental behavioral effects associated with drug abuse. For example, glial cells are markedly affected by exposure to substances of abuse, including opioids, alcohol and psychostimulants (see for review Lacagnina et al. 2017). In addition, accumulating evidence strongly supports that drug-induced alterations in glia physiology within brain regions critically involved in addiction mechanisms, such as the prefrontal cortex, nucleus accumbens, ventral tegmental area, amygdala and hippocampus, might contribute to the vulnerability and persistence of addictive behaviors (Lacagnina et al. 2017; Scofield et al. 2015).

3.1 Effects of chronic THC intake on microglial cells

Microglial cells in resting homeostatic condition express low level of both CB1 and CB2 receptors, while they express CB2 receptors at detectable levels upon activation (Carlisle et al. 2002; Stella, 2010; Walter et al. 2003), suggesting that the endocannabinoid system could be sensitive to changes in glia cell physiology. In particular, several studies provide strong evidence that CB2 receptors are up-regulated primarily on microglial cells upon activation in response to various insults and stimuli (Cabral and Griffin-Thomas, 2009). Furthermore, microglial cells have been shown to produce endocannabinoids at higher levels than neurons in vitro (Walter et al. 2003), suggesting that endocannabinoid production by activated microglial cells could play a pivotal role during neuroinflammation processes. Importantly, the endocannabinoids anandamide (AEA), palmitoylethanolamide (PEA) and 2-

arachidonoylglycerol (2-AG) affect immune function mostly through CB2 receptors (Cabral et al. 2015). Based on these pieces of evidence, it is reasonable to presume that chronic cannabis use might target not only neurons but also microglial cells. Hence, the behavioral outcomes associated with cannabis use could arise as a consequence of abnormal reciprocal interactions between these two cell populations.

The first evidence for an involvement of microglial cells in the effects of cannabis comes from the study by Cutando et al. (2013). These authors demonstrated the involvement of microglial cells in cerebellar conditioned learning (evaluated in the delayed eye-blink conditioning test) and motor coordination deficits induced by sub-chronic THC administration in adult male mice. THC-induced impairment in the learning paradigm was associated with alterations in microglial morphology mainly in the molecular layer of the cerebellum, and with enhanced expression of specific pro-inflammatory genes, such as IL-1 β . Remarkably, microglia activation was associated with cerebellar CB1 receptor downregulation, and a similar neuroinflammatory phenotype was observed in the cerebellum of CB1 $-/-$ mice. Furthermore, all these alterations were region-specific since no changes were present in the hippocampus, striatum or prefrontal cortex. The deficit in cerebellar associative learning and motor coordination in sub-chronically THC-treated mice and in CB1 $-/-$ mice was prevented by pharmacological blockade of microglial activation or IL-1 receptor signaling, thus providing a functional association between THC-induced microglia activation, cerebellar-dependent associative learning and motor impairments. These results reveal the critical role of microglia-mediated signaling in cerebellar dysfunctions triggered by CB1 receptor downregulation. Noteworthy, this new neurobiological mechanism for deleterious effects of THC on cerebellar functions possesses a high translational relevance, since the neuronal circuits involved in the eye-blink conditioning response are the same in mice and humans (Skosnik et al. 2008; Steinmetz et al. 2012).

In line with Cutando's data, Zamberletti et al. (2015) showed a neuroinflammatory profile in the prefrontal cortex of adult female rats exhibiting cognitive impairment and depressive-like behaviors following chronic THC treatment during adolescence. The neuroinflammatory state was characterized by altered microglia morphology, increased expression of the pro-inflammatory markers TNF- α , iNOS and COX-2, and a reduction of the anti-inflammatory cytokine, IL-10. As reported by Cutando and colleagues (2013), THC-induced microglia activation was region-specific since no alterations were detected in the nucleus accumbens, hippocampus and amygdala. Of note, the neuroinflammatory phenotype induced by adolescent THC treatment was associated with down-regulation of CB1 receptors on neuronal cells and a concomitant up-regulation of CB2 receptors on microglial cells within the prefrontal cortex. Interestingly, administration of the pharmacological inhibitor of glia activation Ibudilast during THC treatment attenuated short-term memory impairment present in adult rats, and prevented the increases in TNF- α , iNOS, COX-2 levels as well as the up-regulation of CB2 receptors on microglial cells (Zamberletti et al. 2015). In contrast, neither THC-induced depressive-like behaviors nor neuronal CB1 receptor down-regulation were affected by Ibudilast treatment. The authors outline several provocative possibilities arising from their data:

1. Besides triggering long-term changes in cortical endocannabinoid, glutamate and GABA systems (Rubino et al. 2015; Zamberletti et al. 2014), chronic THC treatment during adolescence, at least in female rats, also prompts immune dysfunction in the prefrontal cortex and these events could potentially act in concert to cause the long-term cognitive impairment associated with the treatment.
2. Modulation of microglia activity can be a potential tool in the prevention of cognitive impairments associated with adolescent THC exposure, thus providing an interesting new possibility for treating cognitive deficits associated with prolonged cannabis consumption.
3. THC can induce a pro-inflammatory or anti-inflammatory picture depending on the status of the brain. In pathological states, THC exerts an anti-inflammatory action (Nagarkatti et al. 2009), whereas a pro-inflammatory role can result from prolonged CB1 receptor stimulation in the healthy brain.

Different results were provided by Lopes-Rodriguez et al. (2014), when examining the effect of adolescent THC treatment in male and female rats at adulthood. In males, an increased proportion of reactive microglial cells was observed in the hilus of the dentate gyrus in the hippocampus, whereas an opposite trend was found in females. THC also reduced immunostaining for CB1 receptors in the hippocampus of females but did not alter CB1 receptor levels in males. Importantly, this study provides evidence for a sex-dimorphism of the effects of chronic THC administration during adolescence on microglia alterations.

3.2 Effects of chronic THC intake on astrocytes

Many studies were able to confirm, both in vitro and in vivo, that astrocytes functionally express CB1 receptors, which are involved in important mechanisms that underlie brain functions (Navarrete and Araque, 2008, 2010; Han et al. 2012; Bosier et al. 2013). In addition, astrocytes can produce endocannabinoids mainly through Ca^{2+} - and ATP-dependent pathways (Stella, 2010) and recent data indicate that astroglial CB1 receptors might control endocannabinoid turnover in the brain (Belluomo et al. 2015); thereby modulating the retrograde neuronal signaling at CB1 receptors.

Only few papers to date assess the consequences of prolonged CB1 receptor stimulation on astrocyte reactivity. In a first study, adolescent THC treatment was shown to increase the levels of the astrocyte marker glial fibrillary acidic protein (GFAP) in the hilus of the dentate gyrus of both male and female rats (Lopes-Rodriguez et al. 2014). More recently, Zamberletti et al. (2016), using the same treatment protocol previously applied to females (Zamberletti et al. 2015), demonstrated that the behavioral phenotype triggered by adolescent THC treatment in male rats overlaps only partially with the one present in females, being characterized by poorer memory performance and psychotic-like behaviors, without alterations in the emotional component that instead were observed in females. Interestingly, when the authors looked at the possible molecular underpinnings of this phenotype, sex-differences were observed, in terms of brain region affected and profile of pro-neuroinflammatory biomarkers. Alterations in astrocyte reactivity were found in the hippocampus after adolescent THC treatment, supported by increased levels of the specific

astrocyte marker GFAP. Astrocyte activation was associated with increased protein expression of the pro-inflammatory mediators TNF- α and iNOS, together with a concomitant reduction of the anti-inflammatory cytokine IL-10. These alterations were paralleled by significant increases in the expression of the NMDA receptor subunit GluN2B, the AMPA subunits GluA1 and GluA2, as well as the pre-synaptic marker synaptophysin and the post-synaptic marker PSD95. The coexistence of synapse and astrocyte alterations in the same brain region appears very intriguing since there is now common agreement that astrocytes are crucially involved in the control of surrounding synapses. Indeed, astrocytes sense neuronal and synaptic activity and this evidence suggests that activated astrocytes, by promoting a pro-inflammatory phenotype, might contribute to the alterations in glutamatergic synapses induced by adolescent THC. Remarkably, distinct from females (Zamberletti et al. 2015), no changes in inflammatory markers were observed in the prefrontal cortex.

Collectively, available data on the effect of chronic THC exposure on microglia and astrocytes suggest an important role played by these cells in response to chronic activation of CB1 receptors, thus strengthening the hypothesis that both cell populations might have an essential role in monitoring synaptic activity (Bilbo and Schwarz, 2012; Graeber, 2012; Haydon et al. 2009; Kettenmann et al. 2013; Schafer and Stevens, 2013). Another important observation from these studies regards the marked sex differences in response to cannabinoid chronic treatment. Indeed, the long-term effect of THC administration on glial cells appears to be both sex- and region-dependent, hippocampus and cerebellum being the most sensitive brain areas in males while cortex is most affected in females. Moreover, both astrocytes and microglia take part in the inflammatory response in the male brain, while mainly only microglial cells seem to be involved in females.

Based on current literature data, we speculate that excess of glutamate in the synaptic cleft resulting from reduced inhibitory control exerted by CB1 receptors at the level of glutamatergic terminals might activate microglia and/or astrocytes (depending upon the brain region and the sex of the animals) that in turn trigger the inflammatory response. The several mediators of this response (i.e. IL-1 β , TNF- α , iNOS) might affect neuronal functionality thus leading to the learning disability described (Cutando et al. 2013; Zamberletti et al. 2015) (Fig. 2).

As the expression of CB1 receptors is higher on GABAergic than on glutamatergic neurons (Marsicano and Lutz, 1999), decreased CB1 receptor expression on GABAergic neurons might also contribute to glia activation following chronic cannabis use. Interestingly, an increase in neuroinflammatory markers has been found in CB1 receptor deficient mice and it was dependent on CB1 receptors in GABAergic neurons (Albayram et al. 2011), suggesting that CB1 receptor activity on GABAergic terminals might regulate the homeostatic balance between pro- and anti-inflammatory processes. Of course, specific studies are needed in order to establish whether reduced CB1 receptor signaling on GABAergic neurons could contribute to neuroinflammation processes associated chronic cannabis exposure.

Further studies are needed to thoroughly comprehend the role of glial cells in mediating the behavioral and synaptic effects of chronic cannabinoid exposure. Advancements in our

ability to control the activity state of these cells should allow us to dissect the contribution of neurons and glia in regulating behavior in health and disease, including drug addiction.

4. Maternal cannabis use disorder and familial transmission of substance use disorders

The main psychoactive ingredient in cannabis, THC, has a long half-life in fat deposits (~8 days) and can long be detected in both blood and urines (~30–40 days) (Khare et al. 2006). THC also readily crosses the fetoplacental barrier (Harbison and Mantilla-Plata, 1972; Hutchings et al. 1989), which results in a prenatal exposure to THC lasting long after the use is discontinued due to slow fetal clearance. In addition, THC can be detected in mother's milk (Astley and Little, 1990; Hutchings et al. 1989; Perez-Reyes and Wall, 1982), thus prolonging the exposure to THC to other sensitive periods of development (Friedrich et al. 2016). Finally, THC levels in cannabis have exponentially increased (~25-fold) since 1970s and 1980s (Mehmedic et al. 2010), and pregnant women also use the illicit marketed synthetic cannabinoids contained in Spice branded products, such as JWH-018 and others (Psychoyos and Vinod, 2013). These latter, similarly to "cannabinoid research chemicals" that fall under the category of "designer drugs" and claim to have cannabis-like effects (Fattore and Fratta, 2011), are more potent than THC (De Luca et al. 2015, 2016; Psychoyos and Vinod, 2013) and might be as harmful as THC to embryonic development and the resulting behavior. All of the abovementioned facts act as risk factors for proper development, as maternal tissues act as reservoirs for THC as well as for other cannabinoids (Friedrich et al. 2016). Consequently, we find a broadening spectrum of interference of exogenous cannabinoids with the roles played by endocannabinoids during ontogeny and throughout development (Alpar et al. 2016; Psychoyos and Vinod, 2013).

4.1 Prenatal cannabis exposure and increased vulnerability to Substance Use Disorders

Cannabis is frequently abused among pregnant women, with reported prevalence rates in developed Western Countries of 5% of all pregnant women (Ebrahim and Gfroerer, 2003; Fergusson et al. 2002), but it is likely that these rates are significantly underestimated. Notably, pregnant women using cannabis do not often discontinue its use/abuse despite their particularly vulnerable "state" (Moore et al. 2010). This persistent behavior is likely due to the widespread acceptance of cannabis as a harmless drug, to the unawareness of potential harm of prenatal THC exposure and of the risks they pose, and finally to the lack of apparent teratogenic effects. Nonetheless, epidemiological evidence points at detrimental postnatal behavioral derangements, which span from neuropsychiatric to behavioral and executive functioning, resulting from early life exposure to cannabis (Alpar et al. 2016; Day et al. 1991; Day and Richardson, 1991; Morris et al. 2011; van Gelder et al. 2009; Vassoler et al. 2013, 2014). Noteworthy, little is known about the implications on human CNS development from the exposure to synthetic cannabinoids, and since our knowledge is only inferred from preclinical studies (Mereu et al. 2003; Psychoyos et al. 2008; Vargish et al. 2016), we can so far predict in humans similar symptoms to those observed following prenatal THC exposure.

Epidemiological longitudinal studies clearly show that prenatal exposure to cannabis produces harmful long-term health effects in the offspring (Calvigioni et al. 2014; Day et al.

1991; Day and Richardson, 1991; Fried and Smith, 2001; Jaques et al. 2014; Morris et al. 2011; van Gelder et al. 2009; Volkow et al. 2014). These include impairments in cognitive processing, such as reduced attention, learning and problem solving, increased impulsivity and engagement in risk-taking behaviors, aggressive and/or addictive behaviors. Notably, considerable evidence has indicated that parental influences, including SUD, play a critical role in the etiology of early- to mid-adolescence substance use (Dishion et al. 1999). Thus, poor parenting, together with the environmental context (including peer environment), both child and social factors, should be considered in order to understand patterns of SUD. More importantly, vulnerability to SUD appears to rely on long-term neurobiological and behavioral consequence of prenatal exposure to drugs of abuse, including cannabis (for review see Jutras-Aswad et al. 2009; Morris et al. 2011) (Fig. 3).

In particular, a significant association has been found between prenatal exposure to cannabis and the initiation and use of cannabis among male adolescents (16–21 year old) (Porath and Fried, 2005). In addition, prenatal exposure to cannabis was found to be a significant predictor of cannabis use at age 14, in terms of both age of onset and frequency of cannabis use, even when other important and influential factors (e.g., mother's socio-economic status, peer drug use) were taken into consideration (Day et al. 2006).

4.2 Cellular and molecular changes following maternal THC exposure and predisposing to Substance Use Disorders

The paucity of studies on molecular changes occurring in the human brain and the inadequate information on their longitudinal impact significantly delay our understanding of neurobiological underpinnings of this risk factor, particularly for SUDs. The importance of identifying and filling this knowledge gap has been recognized, however, the study of neuronal basis of developmental deficits at molecular and circuit level in offspring upon maternal THC exposure is in its infancy. In fact, despite the prominent role played by the endogenous cannabinoid system during ontogeny and in the control of brain maturation (de Salas-Quiroga et al. 2015; French et al. 2015; Galve-Roperh et al. 2013; Harkany et al. 2007; Liang et al. 2014; Vitalis et al. 2008; Wu et al. 2010), as well as the high prevalence of cannabis use among pregnant women, its impact on the developing brain has remained elusive (Jutras-Aswad et al. 2009; Kawash et al. 1980; McBride, 2014; Navarro et al. 1995; Saez et al. 2014; Schneider, 2009; Tortoriello et al. 2014; Vargish et al. 2016). Remarkably, an association between prenatal cannabis exposure and decreased pro-enkephalin mRNA levels in the striatum, increased μ -opioid receptor expression in the amygdala and reduced κ -opioid receptor mRNA levels in the thalamus was found in human fetus (Hurd et al. 2005; Jutras-Aswad et al. 2009; Wang et al. 2006). In addition, maternal CUD decreases expression of dopamine (DA) D2 receptors through epigenetic mechanisms in human offspring amygdala, nucleus accumbens (Wang et al. 2004), and ventral striatum (DiNieri et al. 2011). Notably, given the pivotal role of DA D2 receptors in vulnerability to SUD (Volkow et al. 2007), it is plausible that these molecular changes might contribute to the likelihood that the child/adolescent will develop a SUD. Hence, prenatal THC exposure enhances heroin-seeking profiles in rat offspring (Spano et al. 2007). This effect is associated to biphasic changes in nucleus accumbens preproenkephalin (PENK) mRNA expression levels throughout development. In particular, reduced PENK mRNA expression

levels were observed during early development while elevated PENK mRNA expression levels were found at adulthood. In addition, while no changes in expression were detected in the striatum, an increased expression was found in the central and medial amygdala at adulthood. The increased heroin self-administration behavior was also associated with changes in expression of CB1, DA and glutamatergic receptor genes in the striatum with a resulting altered striatal synaptic plasticity (Szutorisz et al. 2014). Additionally, maternal cannabinoid exposure disrupts endocannabinoid signaling, in particular the temporal dynamics of cortical CB1 receptors, which contribute to control the fasciculation and pallidal targeting of corticofugal axons (Berghuis et al. 2007; Wu et al. 2010; Diaz-Alonso et al. 2012). Prenatal THC induced impairment in the establishment of the corticofugal tract and a cortical reorganization of axonal morphology (Tortoriello et al. 2014). Furthermore, an increased density of CB1 receptor positive boutons was found in the stratum radiatum of the hippocampal CA1 (Tortoriello et al. 2014). Altogether, these effects resulted in an impaired long-term synaptic plasticity in both CA1 stratum radiatum and pyramidal and in an aberrant rewiring of fetal cortical circuitry that might affect computational capabilities of neuronal networks in the offspring (Alpar et al. 2014; de Salas-Quiroga et al. 2015; Saez et al. 2014; Tortoriello et al. 2014). Finally, maternal cannabinoid exposure increases mRNA levels of the neural adhesion molecule L1, a key protein for processes of cell proliferation and migration, neurite elongation and guidance, and synaptogenesis in a sex-dependent manner (Gomez et al. 2003). These effects add to the interference with neurotransmitter synthesis and signaling, with morphogenesis and proper circuitry functioning in the same areas (i.e. amygdala, cortex, dorsal striatum/caudate putamen, hippocampus, mediodorsal thalamus amongst others). In addition, preclinical studies report an impaired DA function in the striatum, substantia nigra and VTA, characterized by an increased tyrosine hydroxylase activity (Bonnin et al. 1995, 1996; Rodriguez de Fonseca et al. 1991) that might contribute to disturb proper neuronal development in the cortex, hippocampus, amygdala and nucleus accumbens. Hence, DA plays a role in activity-dependent changes in synaptic strength by influencing emotional, motivational, cognitive, and motor processes, and is key in SUD. Since endocannabinoids fine-tune DA neuronal excitability and diverse forms of synaptic plasticity at DA cells (Melis and Pistis, 2012; Oleson et al. 2012; Wang and Lupica, 2014; Wenzel and Cheer, 2014), it is plausible that changes in DA release within cortical and subcortical regions not only might alter natural behavior, but it would also attribute motivational salience to otherwise neutral environmental stimuli (Berridge and Robinson, 1998). The abovementioned aberrant effects on structural organization of neuronal networks and synapse re-positioning occurring early on development (Keimpema et al. 2011), which rely on cannabinoid receptor-mediated signaling events (Berghuis et al. 2007; Tortoriello et al. 2014), might confer susceptibility to a variety of neuropsychiatric disorders including an endophenotype of SUD, which is one phenotype observed in the offspring born to cannabis users.

4.3 Prenatal Cannabis exposure, epigenetic inheritance and increased susceptibility to Substance Use Disorders

Early life adversity is a risk factor for the development of behavioral and emotional disorders that can persist through adulthood, and is often transmitted across generations via epigenetic mechanisms. In particular, there is an extensive body of literature documenting

offspring effects following prenatal exposure, even prior to conception (Vassoler et al. 2014; Vassoler and Sadri-Vakili, 2014), to drugs of abuse (Hurd et al. 2014; Szutorisz et al. 2014; Vassoler et al. 2013, 2014; Watson et al. 2015). A detailed analysis of the research relevant to maternal cannabis and the discrete epigenetic mechanisms providing biological underpinnings to cross-generational effects on gene expression and behavior is beyond the scope of this review, and the authors refer to a recent excellent review written by Szutorisz and Hurd (2016). Nonetheless, the observations that many aberrant effects extend into subsequent generations of offspring whose parents were exposed to cannabinoids before mating (Szutorisz et al. 2014; Byrnes et al. 2012; Vassoler et al. 2013; Watson et al. 2015) substantiate the hypothesis that alterations at system level (e.g. glutamatergic, dopaminergic, opioidergic systems) and in synaptic transmission in THC offspring might have trans-generational effects (Szutorisz et al. 2016; Szutorisz and Hurd, 2016). Particularly, epigenetic aberrations influencing the risk and conferring an endophenotype to SUDs can also be inherited through parental germline (Vassoler and Sadri-Vakili 2014, Watson et al. 2015). Hence, such epigenetic changes can be considered as an inheritable factor together with other genetic traits and environmental factors that confer vulnerability to SUD (Feng and Nestler, 2013). In fact, a genome-wide approach identified a series of molecular targets and pathways that are associated to THC cross-generational effects (Watson et al. 2015). In particular, Watson et al. (2015) found changes leading to long-lasting (i.e., cross-generational) DNA methylation alterations in the nucleus accumbens of adult progeny that were exposed to THC. Remarkably, they found significant enrichments of differentially methylated regions (DMRs) within loci involved in a range of GO terms including genes relevant to behavioral and physiological traits characteristics of prenatal THC-exposed phenotype (Szutorisz et al. 2014). To our knowledge, this study (Watson et al. 2015) provides the first evidence on cross-generational epigenomic perturbations in the nucleus accumbens that are associated with THC exposure, and include DMRs localized to genes that are key components of SUD-related traits (i.e. dopamine-glutamate interactions). Noteworthy, these effects on genes that regulate synaptic plasticity and glutamatergic transmission might be revealed only under particular circumstances such as, for instance, in conjunction with the presence of regulatory proteins, or at specific developmental stages, or in response to certain stimuli. Hence, disease-associated methylation alterations might exert effects during early developmental periods before onset of the disease. However, it is worth to remark that SUD, as a complex psychiatric disorder, also depends upon the interaction among environmental factors and other biological factors that might indeed prevent its clinical manifestation.

The understanding of the heritability of epigenetic marks in relation to the consequences of parental THC exposure is still nowadays an understudied public health question. Nonetheless, the number of clinical and preclinical studies on long-term detrimental effects of maternal cannabis on offspring, as well as the following generations, are solid and constantly increasing. Gaining knowledge on when and where maternal cannabis exposure sets into motion epigenetic changes contributing to long-term effects in mesocorticolimbic gene regulation is paramount in order to identify a therapeutic target. Equally important, preclinical studies that increase our knowledge on the impact of gestational exposure to synthetic cannabinoids, such as those found in Spice branded products, on neurotransmitter

signaling, neuronal development, and resulting behaviour in the offspring are needed. Finally, unveiling mechanisms and links between gene expression impairments and endophenotypes might be useful for developing prevention strategies and tailored therapeutic interventions.

5. Conclusions

Overall, the recent findings discussed in this review provides novel insights into the neurobiology underpinning long-term consequences of cannabis use. Moreover, the novel experimental paradigm of cannabis self-administration in rodents here proposed will boost research in the field of cannabis addiction, hopefully leading to the development of treatments for CUD in the next decade.

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Figure 1. Sample data from the rat model of THC + CBD self-administration and cue- or context-induced drug-seeking

Rats were exposed to 5 days of THC + CBD vapor prior to initiating intravenous THC + CBD daily self-administration sessions. Additionally, rats were food-trained in the absence of cues for a 1 hr prior to beginning self-administration. A 20 sec time out period was used and each infusion was associated with a 2 sec light and tone combined cue. **A)** Responses on the active and inactive levers, and number of drug infusions during the self-administration protocol. **B)** N=8 rats that underwent 10 days of abstinence prior to initiating context extinction training. The data shown are the active and inactive lever presses initiated during the first day of extinction training. Data are shown as mean $\pm$ sem and were analyzed using a Mann-Whitney U test, $p = 0.010$. **C)** Active and inactive lever pressing during context extinction training. **D)** N=6 rats went directly into extinction training for 10 days. These extinction and reinstatement data were pooled with the data from the animals shown in Panel C that were extinguished and cue-reinstated after the context-induced drug seeking session.

Data are shown as mean $\pm$ sem and statistically analyzed using a two-way repeated measures ANOVA. Lever $F_{(1,13)} = 8.63$, $p = 0.012$; Ext vs Cue $F_{(1,13)} = 9.28$, $p = 0.009$; Interaction $F_{(1,13)} = 5.82$, $p = 0.031$. * $p < 0.05$, comparing all groups to active lever pressing induced by context (panel B) or cue (panel D).

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Figure 2. Schematic hypothesis of glial activations following chronic THC treatment at the "quadpartite" synapse

Overactivation of CB1 receptors on synaptic terminals might lead to CB1 receptor downregulation and/or desensitization that is compensated during the treatment but is unmasked when treatment is discontinued. In this specific withdrawal window, the decreased control exerted by CB1 receptors on the release activity at the level of glutamatergic terminals might produce an extended presence of glutamate in the synaptic cleft. Increased glutamate release activates mGluRs associated with the postsynaptic membrane, leading to formation and release of endocannabinoids from the postsynaptic terminal (Hashimoto et al. 2008). Endocannabinoids released from pyramidal neurons, acting through CB1 receptors on astrocytes, can increase astrocyte Ca²⁺ levels stimulating the release of glutamate from these cells (Navarrete et al. 2014), thus contributing/sustaining excitotoxicity. In addition, increased synaptic glutamate activates glutamate (AMPA) receptors on microglial cells, promoting microglia activation and IL-1 β /TNF- α release (Domercq et al. 2013) that might contribute to the learning disabilities associated with chronic THC intake (Cutando et al. 2013; Zamberletti et al. 2015). Remarkably, activated microglial cells rapidly overexpress CB2 receptors (Carlisle et al. 2002; Stella, 2010; Walter et al. 2003), whose stimulation has been shown to modulate microglial reactivity, chemotaxis, proliferation, phagocytosis, migration, promoting neuroprotection (Carrier et al. 2004; Dirikoc et al. 2007; Eljaschewitsch et al. 2006; Ramirez et al. 2005; Walter et al. 2003). Thus, the overall effect of stimulating CB2 receptors via enhancement of endocannabinoid signaling or exogenous agonists would dampen microglia activation or skew microglial cells towards a neuroprotective phenotype (Navarro et al. 2016).

Figure 3. Diagram illustrating the risk factors leading to an at-risk endophenotype for SUD

The interaction among biological make up of the individual, environment (e.g. one or both parental SUD, parental neglect, peer influence, etc) and age-related effects of indirect (i.e. pre/peri-natal exposure to drugs of abuse such as cannabis derivatives) and direct (early onset SUD, including CUD) results in epigenetic modifications and changes at cellular and synaptic level that contribute to the development of an at-risk endophenotype for SUD.