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Review

Cannabis and the developing brain: Insights from behavior

Viviana Trezza [a,*], Vincenzo Cuomo [b], Louk J.M.J. Vanderschuren [a]

[a] Rudolf Magnus Institute of Neuroscience, Department of Neuroscience and Pharmacology, University Medical Center Utrecht, Utrecht, The Netherlands
[b] Department of Human Physiology and Pharmacology, University of Rome "Sapienza", Rome, Italy

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ABSTRACT

The isolation and identification, in 1964, of delta-9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, opened the door to a whole new field of medical research. The exploration of the therapeutic potential of THC and other natural and synthetic cannabinoid compounds was paralleled by the discovery of the endocannabinoid system, comprising cannabinoid receptors and their endogenous ligands, which offered exciting new insights into brain function. Besides its well-known involvement in specific brain functions, such as control of movement, memory and emotions, the endocannabinoid system plays an important role in fundamental developmental processes such as cell proliferation, migration and differentiation. For this reason, changes in its activity during stages of high neuronal plasticity, such as the perinatal and the adolescent period, can have long-lasting neurobehavioral consequences. Here, we summarize human and animal studies examining the behavioral and neurobiological effects of in utero and adolescent exposure to cannabis. Since cannabis preparations are widely used and abused by young people, including pregnant women, understanding how cannabinoid compounds affect the developing brain, leading to neurobehavioral alterations or neuropsychiatric disorders later in life, is a serious health issue. In addition, since the endocannabinoid system is emerging as a novel therapeutic target for the treatment of several neuropsychiatric diseases, a detailed investigation of possible adverse effects of cannabinoid compounds on the central nervous system (CNS) of immature individuals is warranted.

Contents

1. Introduction

Ten years ago, the British newspaper *The Independent* launched a campaign to decriminalise cannabis (Boycott, 1997), considering it a relatively harmless "soft" drug. This campaign culminated in a pro-

* Corresponding author. Rudolf Magnus Institute of Neuroscience, Dept. of Neuroscience and Pharmacology, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands. Tel.: +31 30 2538844 (as of 1 January 2008: +31 88 7568844); fax: +31 30 2538155 (as of 1 January 2008: +31 88 7568155).
E-mail address: vtrezza@umcutrecht.nl (V. Trezza).

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cannabis march of 16,000 people to London's Hyde Park, that led the British Government to downgrade the legal status of the drug. A few months ago, the same newspaper, with "Cannabis: an apology" as front-page headline (Owen, 2007), reversed its landmark campaign for cannabis use to be decriminalised because, as it stressed, there is increasing evidence that cannabis is far from harmless. The newspaper reported record numbers of British teenagers requiring drug treatment as a result of smoking highly potent cannabis strains that are 25 times stronger than the cannabis strains sold a decade ago. With its "apology", *The Independent* reflects the strong, current debate on the health consequences of cannabis use, and highlights two important recent trends in cannabis consumption: first, the availability of more potent varieties of cannabis, termed sinsemilla or skunk; second, the increasing popularity of cannabis among young people, which makes cannabis the world's third most popular recreational drug, after alcohol and tobacco (NIDA, 2005).

In recent years, the exceptional blooming of research around cannabis has aided the understanding of its effects on the brain, and a number of reports have claimed cannabis derivates as novel, promising therapeutic tools for a variety of pathological conditions (Di Marzo et al., 2004; Lambert and Fowler, 2005; Mackie, 2006a; Pacher et al., 2006). At the same time, more and more studies have quantified the extent of the risks of short- and long-term cannabis use (Di Forti et al., 2007; Grotenhermen, 2007; Moore et al., 2007), and cannabis consumption by young people has become a serious health issue.

In humans, cannabis use peaks between 15 and 30 years of age, although there is an emerging trend for continued cannabis consumption by people aged 30–40 years (NIDA, 2005). This pattern of use potentially exposes the developing brain to cannabis during two critical developmental periods. First, in offspring of cannabis-using mothers during the perinatal period. Cannabis preparations are indeed among the illicit drugs most widely abused by pregnant women in Western societies (Fried and Smith, 2001; NIDA, 2005). Since the psychoactive ingredients of cannabis can cross the placenta and be secreted in maternal milk (Hutchings et al., 1989; Jakubovic et al., 1977), understanding whether developmental exposure to cannabis derivates might interfere with the rigidly ordered temporal sequence of events that occur during the ontogeny of the central nervous system (CNS) represents an urgent and exciting challenge. Second, the adolescent brain, undergoing its final development and maturation, may also be exposed to exogenous cannabinoids through use at that particularly sensitive age.

The CNS develops over a long period of time extending from the embryonic stage through adolescence until adulthood, with both synaptogenesis and myelination continuing from the perinatal period through puberty in both animals and humans (Spear, 2000). Thus, given the increasing abuse of cannabis among young people, research into the long-lasting neurobehavioral effects of adolescent cannabis use is warranted.

In this review, we outline recent research into the endocannabinoid system, discussing how cannabinoid compounds may interfere with neuronal signalling and interact with other neurotransmitter systems, such as the endogenous opioid system. We then focus on the role of the endocannabinoid system in brain development and discuss the behavioral and neurobiological effects of cannabinoid exposure during the perinatal and adolescent periods, as highlighted by both human and animal studies. Particular emphasis will be given to the long-term psychiatric implications of cannabinoid exposure during these critical stages of brain development.

2. The endocannabinoid system

The powerful effects of cannabis on the brain have been known for thousands of years (Mechoulam, 1986; Russo, 2007). A variable, subjective combination of euphoria and relaxation, mood changes and altered perception, loss of motor coordination and impaired attention, distorted perception of time and hallucinations, has lead different societies to regard cannabis as an ideal remedy for everyday pains, or as a harmful poison that induces madness. While the psychoactive properties of cannabis have long been recognized, research into its active chemical constituents turned out to be more time consuming than expected (Mechoulam and Hanus, 2000).

Although chemical studies of the constituents of cannabis started in the early 1800s, the most important page in the field of cannabinoid research was not written until 1964 by Gaoni and Mechoulam, who were the first to identify delta-9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis (Gaoni and Mechoulam, 1964).

However, it took another quarter of a century until the cellular target of THC was identified. During this period, the debate between scientists was open. Unlike morphine, cocaine, and other alkaloids of plant origin, THC is a highly hydrophobic compound, a property that erroneously suggested to most researchers that the drug's main effect was to modify the fluidity of cell membranes in a non-selective manner, rather than to activate a selective cell-surface receptor. At the same time, the development of new classes of potent and stereoselective THC analogues and standard radioligand binding techniques strongly supported the existence of specific and high-affinity brain binding sites for THC. An important clue to the existence of cannabinoid receptors was provided by studies showing that incubation of neuroblastoma cells with cannabinoids induced a decrease of cAMP in the cells (Howlett and Fleming, 1984), suggesting that the putative cannabinoid receptors were negatively coupled to adenylyl cyclase. The existence of cannabinoid receptors was confirmed in 1988, when the synthetic cannabinoid CP-55,940 was used as the first probe of cannabinoid receptors by competitive binding assays (Devane et al., 1988). In 1990, the CB_1 cannabinoid receptor was cloned from rat brain (Matsuda et al., 1990), and its immune system counterpart, the CB_2 cannabinoid receptor, was identified by sequence homology 3 years later (Munro et al., 1993).

Autoradiographic and immunohistochemical studies revealed that the CB_1 cannabinoid receptor is the most abundant G-protein-coupled receptor in the brain, particularly expressed in the hippocampus, the cerebellum, the basal ganglia, the cerebral cortex and the amygdala (Herkenham et al., 1990), a distribution pattern which accounts for the cognitive, affective, and motor effects of cannabimimetic compounds. Although their presence has been reported in the CNS, for example in the cerebellum and restricted areas of the brainstem (Onaivi et al., 2006; Van Sickle et al., 2005), CB_2 cannabinoid receptors are mainly expressed in cells of the immune system.

The discovery of cannabinoid receptors launched a new, exciting search: do mammalian tissues also produce cannabinoid-like receptor agonists, or are these receptors targeted only by plant cannabinoids and their synthetic analogues?

The previous experience of Pert and Snyder, who first identified opioid receptors in the brain in 1973 (Pert and Snyder, 1973), and Kosterlitz and Hughes, who reported the existence of an endogenous morphine-like substance 2 years later (Kosterlitz and Hughes, 1975), led scientists to the idea that that cannabinoid receptors were not present in the brain just because of some psychotomimetic plant constituents, but had to be activated by specific endogenous ligands. And, just as morphine led to the discovery of the endogenous opioid systems in the brain, THC and its synthetic analogues led, in the end, to the discovery of endogenous agonists of cannabinoid receptors, the so-called "endocannabinoids". In 1992, Devane and coworkers isolated the first endocannabinoid, arachidonoylethanolamide, from the porcine brain, and named it anandamide, referring both to the Sanskrit word "ananda", meaning bliss, and to "amide" for the chemical nature of the compound (Devane et al., 1992). A second endocannabinoid, 2-arachidonoylglycerol (2-AG), was discovered in 1995 (Mechoulam et al., 1995), and numerous endogenous

compounds soon followed, sharing with anandamide the ability to bind and activate at least one cannabinoid receptor.

Due to their lipophilic nature, endocannabinoids are not stored in synaptic vesicles, but are synthesized by neurons following membrane depolarization and increased intracellular Ca^{2+} levels (Freund et al., 2003; Piomelli, 2003). Once released, the newly synthesized endocannabinoids travel in retrograde direction toward the synaptic cleft. Here, they bind to CB_1 cannabinoid receptors on presynaptic terminals (Freund et al., 2003). Activation of CB_1 cannabinoid receptors, in turn, initiates closure of Ca^{2+} channels, opening of K^+ channels, inhibition of adenylyl cyclase activity and stimulation of kinases that phosphorylate tyrosine, serine and threonine residues in proteins. The inhibition or activation of ion channels is one of the primary consequences of activation of CB_1 cannabinoid receptors (Szabo and Schlicker, 2005). Through this influence on ion channels, endocannabinoids can inhibit neurotransmitter release from axon terminals, thus playing a major role in several forms of both short- and long-term synaptic plasticity (Chevaleyre et al., 2006; Mackie, 2006b).

Clearance of anandamide and 2-AG from the synaptic cleft is then rapidly accomplished via a high-affinity, selective, saturable, temperature-dependent process, suggesting carrier-mediated transport (Beltramo et al., 1997). Once inside the cell, anandamide is cleaved by fatty acid amide hydrolase (FAAH), a membrane-bound intracellular serine hydrolase (Cravatt et al., 1996; Hillard et al., 1995; Ueda et al., 1995), whereas 2-AG is metabolized by monoglyceride lipase (MGL), a cytosolic serine hydrolase (Dinh et al., 2002; Goparaju et al., 1999).

Overall, an interesting aspect of endocannabinoid activity appears from this picture. The rapid induction of endocannabinoid synthesis, receptor activation and degradation suggests that these compounds act in the brain primarily as neuromodulators, rather than classical neurotransmitters. Thus, endocannabinoids have been suggested to act "on demand", with a highly regulated, spatiotemporal specific pattern, according to "where" and "when" they are needed (Piomelli, 2003).

2.1. Endocannabinoid-mediated synaptic plasticity

Endocannabinoid-mediated short-term synaptic plasticity includes two electrophysiological phenomena, depolarization-induced suppression of inhibition and depolarization-induced suppression of excitation. The former is due to presynaptic inhibition of GABA release, while the latter results from presynaptic inhibition of glutamate efflux (Chevaleyre et al., 2006; Mackie, 2006b). Interestingly, simultaneous inhibitory effects of endocannabinoids on antagonistic components of functional neurotransmitter systems can be easily found. For example, endocannabinoids inhibit both glutamatergic and GABAergic input to dopaminergic neurons located in the ventral tegmental area, so that the final outcome will depend upon the relative level of activation of these pathways under different behavioral circumstances (Gardner, 2005; Lupica et al., 2004).

The endocannabinoid system is also involved in long-term forms of synaptic plasticity (Carlson et al., 2002; Chevaleyre et al., 2006; Mackie, 2006b). Long-term potentiation is a long-lasting increase in synaptic strength, while long-term depression is a long-lasting weakening of synaptic strength. Both are mechanisms of synaptic plasticity that can persist for hours to weeks and have important implications on various forms of learning and memory. Endocannabinoid-induced long-lasting inhibition of neurotransmitter release has been found in diverse brain structures, at both excitatory and inhibitory synapses (Chevaleyre et al., 2006; Mackie, 2006b).

In contrast to the subtle, physiological functions of endocannabinoids, acute administration of exogenous cannabinoids can strongly disrupt neuronal signalling. A clear example of the difference between the physiological role of the endocannabinoid system, on the one hand, and the pathophysiological consequences of exogenous cannabinoids, on the other, is provided by hippocampal cognitive pathways. At CA3–CA1 synapses in the hippocampus, endocannabinoids facilitate memory encoding (Carlson et al., 2002; Hampson and Deadwyler, 1999). When depolarized, hippocampal pyramidal neurons synthesize endocannabinoids, which travel backwards across the synapse and bind to presynaptic CB_1 cannabinoid receptors; the result is long-term depression of GABA and cholecystokinin (CCK) inputs to the pyramidal cells. By weakening GABA-mediated inhibition, this form of depolarization-induced suppression of inhibition amplifies the activity of neighbouring glutamatergic terminals, thus facilitating the induction of long-term potentiation in individual pyramidal neurons; this might contribute, in turn, to the formation of hippocampus-dependent learning (Chevaleyre and Castillo, 2004). At the same time, CB_1 cannabinoid receptors are also expressed on hippocampal glutamatergic terminals (Katona et al., 2006), although at lower levels than on GABA interneurons (Kawamura et al., 2006). CB_1 cannabinoid receptors on glutamatergic axons serve as a self-limiting feedback loop: prolonged depolarization releases additional endocannabinoids, that terminate further glutamatergic activity and prevent the progression to excitotoxicity (Panikashvili et al., 2001). In contrast to the cognitive-enhancing role of local hippocampal endocannabinoid neurotransmission, exogenous cannabinoid drugs have well-known, deleterious effects on mnemonic processes, due to circuit-independent activation of CB_1 cannabinoid receptors in the hippocampus and other brain areas, with subsequent decrease of synchronized neuronal firing and long-term potentiation (Ameri, 1999; Hampson and Deadwyler, 1999).

Since endocannabinoid-dependent synaptic plasticity has been identified in several brain areas, targeting the endocannabinoid system has emerged as a novel strategy to adjust the threshold for emotional salience, cognitive flexibility, motor fluidity and sensory integration. In this context, current pharmacological approaches include: first, receptor-specific ligands, i.e. direct agonists and antagonists that indiscriminately bind CB_1 cannabinoid receptors throughout the brain (Pertwee and Ross, 2002); second, endocannabinoid inactivation inhibitors, also called "indirect agonists", which interfere with endocannabinoid deactivation and increase endocannabinoid neurotransmission only in active synapses (Freund et al., 2003; Piomelli et al., 2006). By preserving the spatiotemporal specificity of endocannabinoid-mediated fine-tuning of synaptic activity, indirect cannabinoid agonists prolong and magnify endocannabinoid signalling, and are therefore expected to have minimal generalized side-effects, in contrast to direct full agonists that produce non-selective CB_1 cannabinoid receptor stimulation in multiple brain areas.

2.2. Interaction between endocannabinoid and opioid neurotransmission

Opioid and cannabinoid receptors are co-expressed in several brain regions and share several common characteristics: they are both coupled to $G_{i/o}$-GTP-binding proteins that inhibit adenylyl cyclase activity, block voltage-dependent Ca^{2+} channels, activate K^+ channels and stimulate the MAP kinase cascade. Both receptors are mainly located presynaptically, and their activation causes inhibition of neurotransmitter release (Vigano et al., 2005). These similarities are significant, because opioid and endocannabinoid neurotransmission mediate overlapping pharmacological responses in clinically important areas, such as drug abuse and pain management (Manzanares et al., 1999). Thus, cannabinoid effects, mediated by CB_1 cannabinoid receptors, can be modulated by opioid antagonists, and vice versa (Fattore et al., 2005). The precise nature of this interaction differs as a function of the endpoint measured (e.g., analgesia, dependence, reinforcement) and the species tested. However, although bidirectional interactions between endocannabinoid and opioid neurotransmission are widely reported, the underlying mechanisms are as yet poorly understood.

It has been proposed that cannabinoids may increase the synthesis or release of endogenous opioids, and vice versa (Corchero et al.,

1997a,b; Kumar et al., 1990; Manzanares et al., 1998). Among the abundant evidence supporting this hypothesis are studies demonstrating that (1) THC increases the expression of opioid peptide precursors (prodynorphin and proenkephalin) in the spinal cord, and proopiomelanocortin in the hypothalamus (Corchero et al., 1997a,b); (2) administration of the CB_1 cannabinoid receptor agonist CP 55,940 through a spinal catheter enhances the release of dynorphin B concurrent with antinociceptive effects in rats (Houser et al., 2000); (3) perinatal (Kumar et al., 1990) and adolescent (Ellgren et al., 2007) cannabinoid exposure induces long-lasting functional effects on the endogenous opioid system, such as changes in the levels of met-enkephalin and β-endorphin; (4) THC increases the release of endogenous enkephalins in the nucleus accumbens of awake, freely moving rats (Valverde et al., 2001). In this view, one possible mechanism underlying cannabinoid–opioid interactions in the modulation of reward processes might be that cannabinoid receptor activation changes the levels of endogenous peptides in mesolimbic areas that, in turn, influence dopaminergic activity.

Alternatively, like other G-protein-coupled receptors, cannabinoid and opioid receptors might be physically associated to form heterodimers. Although this fascinating hypothesis has not been confirmed by co-immunoprecipitation experiments yet, several findings indirectly support this possibility. Recently, Rios et al. (2006) showed that coactivation of μ-opioid and CB_1 cannabinoid receptors results in attenuation of signalling by either receptors. In line with this finding, Schoffelmeer et al. (2006) provided pharmacological evidence for allosterically interacting μ-opioid and CB_1 cannabinoid receptors in the nucleus accumbens core. The evidence that in μ-opioid receptor knockout mice the signalling strength of CB_1 cannabinoid receptor agonists is significantly reduced (Berrendero et al., 2003) also argues for the existence of physically-associated opioid–cannabinoid hetero-oligomers. More evidence is required before the heterodimer interpretation of cannabinoid–opioid interaction can be fully accepted. If direct interaction of μ-opioid and CB_1 cannabinoid pharmacophores in a quaternary complex indeed exists, it might provide real benefits by opening the potential for development of new ligands able to target systems that coexpress CB_1 cannabinoid and μ-opioid receptors.

2.3. The endocannabinoid system and brain development

2.3.1. Ontogeny of the endocannabinoid system

The existence of several components of the endocannabinoid system has been demonstrated in the fetal and neonatal rat brain (Fernandez-Ruiz et al., 2000). The endocannabinoid system shows significant differences in the expression and activity of its components during consecutive developmental phases.

CB_1 cannabinoid receptor binding and mRNA levels can be detected around gestational day 11–14 in rats, coinciding with the time of phenotypic expression of most neurotransmitters. At these fetal ages, cannabinoid receptors appear to be functional, since they are already coupled to signal transduction mechanisms that involve GTP-binding proteins (Berrendero et al., 1998). The levels of these receptors are substantially higher than those seen in the adult rat brain (Berrendero et al., 1999). Moreover, in the fetal and early neonatal rat brain, there is an atypical distribution of CB_1 cannabinoid receptors compared to the adult brain, particularly with regard to the location of receptor binding in white-matter areas (Romero et al., 1997) and mRNA expression in subventricular zones of the forebrain (Berrendero et al., 1998, 1999), areas in which these receptors are scarce or undetectable in the adult brain.

With regard to humans, CB_1 cannabinoid receptors are detected in week 14 of gestation in the hippocampus, and by week 20 in the amygdala (Wang et al., 2003). As in animal models, high density of CB_1 cannabinoid receptors has been detected during human prenatal development in white-matter areas that are practically devoid of these receptors in the adult brain. This atypical distribution of CB_1 cannabinoid receptors, which is similar to that observed in rats, has been interpreted, for both species, as indicating a specific role for the endocannabinoid system in several developmental events, such as metabolic support, cell proliferation and migration, axonal elongation, and later, synaptogenesis and myelogenesis (Fernandez-Ruiz et al., 2000). This hypothesis is supported by recent findings demonstrating an important role of endocannabinoids as axon guidance cues in different neuronal populations, such as cortical CB_1-expressing GABAergic interneurons in rodents and *Xenopus* spinal cord neurons (Berghuis et al., 2007). Support also comes from the demonstration of a role for CB_1 cannabinoid receptors in neurite remodeling in vitro (Zhou and Song, 2001), and from findings showing that endocannabinoids inhibit both cortical neuron differentiation to mature neurons using in vitro cellular models and adult hippocampal neurogenesis in vivo (Rueda et al., 2002).

Concerning the ontogeny of endocannabinoid ligands, both anandamide and 2-AG are present in the fetal rat brain at gestational day 21 (Berrendero et al., 1999). However, the amount of 2-AG at this developmental age is significantly higher than that of anandamide and peaks at postnatal day (PND) 1, with values twofold higher than those found at other ages. In contrast, anandamide levels increase during the early postnatal period, reaching their maximum in the adolescent brain (Berrendero et al., 1999). The higher levels of 2-AG in the fetal rat brain might indicate a more important role for 2-AG than anandamide as an endogenous ligand for the CB_1 cannabinoid receptor during brain development. However, the increase of 2-AG observed at PND 1 might be related to an increase in the formation of diacylglycerol, which is an intermediate in the synthesis of 2-AG. Diacylglycerol has indeed been reported to be significantly involved in the metabolism of phosphoglycerides and sphingolipids during neurite formation and myelinogenesis, and hence neural development (Araki and Wurtman, 1997; Sillence and Allan, 1998).

2.3.2. Effects of cannabinoids on neurotransmitter maturation

There is a large body of evidence that exposure to cannabinoids during critical periods for brain maturation can affect the development of several neurotransmitter systems. In particular, several studies have demonstrated effects of cannabinoids on the maturation of catecholaminergic (Fernandez-Ruiz et al., 2000; Garcia-Gil et al., 1997; Hernandez et al., 2000), serotonergic (Molina-Holgado et al., 1997, 1996), GABAergic (Garcia-Gil et al., 1999), glutamatergic (Suarez et al., 2004) and opioid systems (Fernandez-Ruiz et al., 2004; Kumar et al., 1990; Vela et al., 1998; Wang et al., 2006).

The effects of cannabinoids on the development of catecholaminergic pathways appear before the complete differentiation and maturation of these projections into their target areas, in particular during the final part of gestation, when cannabinoids are able to affect the expression of key genes for catecholaminergic transmission, such as tyrosine hydroxylase (TH) (Bonnin et al., 1996). TH appears around gestational day 14 in rats and plays an important role in axon guidance and synaptogenesis (Bonnin et al., 1996). In rats, perinatal exposure to THC has been found to cause a marked rise in TH gene expression in fetal brain at gestational day 14, together with a pronounced increase in the levels and activity of this enzyme (Bonnin et al., 1996). Moreover, cultured neurons obtained from fetuses exposed to THC daily from gestational day 5 exhibited higher TH activity compared to cells obtained from vehicle-exposed fetuses (Hernandez et al., 2000). These data suggest that interference of plant-derived cannabinoids with the events involving the expression of TH gene during brain development might contribute to the abnormal pre- and postnatal maturation of TH-containing neurons and their related targets.

Molina-Holgado et al. (1997, 1996) observed changes in the development of the serotonergic system in rats perinatally exposed to THC. In particular, they found a decrease in serotonin content at birth in diencephalic areas but not in other brain regions (Molina-Holgado et al., 1996), and reduced serotonin and increased 5-hydroxy-

indoleacetic acid contents in the hypothalamus, neostriatum, hippocampus, septum nuclei and midbrain raphe nuclei of adults rats perinatally exposed to THC (Molina-Holgado et al., 1997).

Garcia-Gil et al. (1999) observed that perinatal cannabinoid exposure did not produce any measurable effects in the content of GABA and in the activity of glutamic acid decarboxylase (GAD) in motor and limbic regions of the adult rat brain. However, both adult males and females, that had been perinatally exposed to THC, exhibited a higher responsiveness to GABA B receptor agonists, such as baclofen. This is in concordance with the predominant role proposed for GABA B over GABA A receptors in the interaction between the GABAergic and the endocannabinoid systems in the adult brain (Romero et al., 1996).

The glutamatergic system appears to be critically affected by early cannabinoid exposure. Developmental THC exposure induced a decrease in the expression of glutamate receptors, which might lead to functional alterations through the inhibition of glutamatergic neurotransmission (Suarez et al., 2004).

A reduction in glutamate release was observed in hippocampal cell cultures obtained from pups born from mothers exposed to the synthetic CB_1 cannabinoid receptor agonist WIN55,212-2, as well as in the hippocampus and cerebral cortex of juvenile and adult rats born from WIN55,212-2-treated dams (Antonelli et al., 2004; Mereu et al., 2003). The reduction in the number of cortical neurons and the disturbance in neurite outgrowth induced by prenatal exposure to WIN55,212-2 (Antonelli et al., 2005) suggest that these alterations might represent a possible mechanism underlying the decrease in glutamate levels induced by exposure to cannabinoid drugs during development. In addition, it has recently been shown that prenatal WIN55,212-2 exposure increases expression and functional activity of glutamate transporters in the prefrontal cortex of adolescent rats, an effect which may also contribute to the observed decrease of cortical glutamate outflow induced by cannabinoid compounds (Castaldo et al., 2007). Consistent with data obtained with WIN55,212-2, perinatal exposure to THC induced an enduring alteration in the cortical expression of genes related to glutamatergic neurotransmission, associated with a long-lasting decrease in cortical extracellular glutamate levels (Campolongo et al., 2007).

Exposure to cannabinoids during early developmental periods alters the normal maturation of opioid neurons. For example, it has been reported that cannabinoid administration increases proenkephalin-mRNA levels in rat fetuses at gestational day 16 and 18 in motor, limbic and diencephalic structures (Perez-Rosado et al., 2000). Adolescent exposure to cannabinoid compounds has also been shown to affect the postnatal development of opioid neurons (Ellgren et al., 2007). These alterations in opioidergic neurotransmission are likely to produce important long-lasting functional changes in these neurons in the adult brain (Ramos et al., 2005). Indeed, it has been found that adult animals that had been exposed to cannabinoids during critical stages of brain development exhibit alterations in neuroendocrine control (Kumar et al., 1990), pain sensitivity (Vela et al., 1995), and reward processes (Ellgren et al., 2007; Gonzalez et al., 2003; Singh et al., 2006; Spano et al., 2007; Vela et al., 1998).

3. Behavioral consequences of cannabinoid exposure during pregnancy and/or lactation

3.1. Neurobehavioral teratology

Although maternal exposure to high doses of alcohol, tobacco, marijuana, opiates and cocaine results in both impaired growth and several abnormalities in the fetus, moderate drug use during pregnancy also has subtle, long-lasting postnatal consequences, manifested as alterations in behavior and cognition. Research into the postnatal consequences of prenatal smoking, drinking or cannabis use utilizes the concepts and methods of behavioral teratology (Vorhees, 1989). Behavioral teratology investigates whether prenatal or postnatal exposure to a physical or a chemical agent induces significant changes in neurobehavioral development (Coyle et al., 1976).

Whereas the thalidomide tragedy may be considered the major stimulus for research in general teratology, ethanol can be considered the primary stimulus for research in behavioral teratology. Since Jones and Smith published their paper describing the Fetal Alcohol Syndrome (Jones and Smith, 1973), there has been a dramatic increase in the number of clinical reports examining the neurobehavioral and cognitive consequences in children exposed to a wide variety of drugs during prenatal development (Fried, 2002).

Over the years, teratological investigators have demonstrated that agents that are relatively harmless to the mother may have significant negative consequences to the fetus. Vorhees (1989) has modified and extended general teratological principles to behavioral teratology, resulting in two major postulates: (1) vulnerability of the CNS to injury extends throughout the fetal and neonatal periods and beyond infancy, including all aspects of development of the nervous system (e.g. neurogenesis, neuronal differentiation, arborization, synaptogenesis, functional synaptic organization, myelination, gliogenesis, glial migration and differentiation), and (2) the most frequent manifestation of injury to the developing CNS does not result in nervous system malformations, but rather in functional abnormalities that may not be detectable at birth.

Substances that are most frequently abused by pregnant women include nicotine, alcohol, and cannabis. Although there is a quite extensive body of literature on birth and behavioral effects in newborns and infants after prenatal exposure to maternal smoking, drinking and, to a lesser extent, cannabis use, information on neurobehavioral and cognitive teratogenic findings beyond these early ages is still quite limited. Furthermore, most studies have focused on prenatal exposure to heavy levels of smoking, drinking or cannabis use, whereas there is scarce information about the long-term behavioral consequences of exposure to moderate doses of these substances.

One of the strategies for studying the developmental effects of drugs of abuse is the use of animal models. They allow the strict monitoring of the influence of confounding variables present in human studies, such as dosage, number of drugs used, stage of pregnancy, timing of drug exposure, drug-induced alterations in maternal appetite or the influence of social problems typically associated with drug use. Although the course of fetal development, drug metabolism or the pattern of drug use in humans does not closely resemble that of laboratory animals, the information obtained in these models is crucial for understanding the biological mechanisms underlying drug effects, and is of great help for designing prospective studies in humans (Huizink and Mulder, 2006).

3.2. Human studies

Cannabis preparations are among the illicit drugs most commonly used by pregnant women in Western countries (Fried, 2002). In spite of the obvious importance of investigating the long-term behavioral consequences of in utero exposure to cannabis, scientific data on this issue are surprisingly scarce.

There are only two extended longitudinal cohort studies, initiated in 1978 and 1982, that have documented the neurobehavioral and developmental effects of prenatal exposure to cannabis past early school age. The Ottawa Prenatal Prospective Study (OPPS) examined the consequences of cannabis use and smoking during pregnancy in a sample of low-risk, white, predominantly middle-class families, as yet up to the age of 18–22 years (Fried, 2002). The Maternal Health Practices and Child Development Study (MHPCD) examined a high-risk cohort of low socioeconomic status, started in 1982 at Pittsburgh, and has focused on the consequences of prenatal use of cannabis, alcohol and cocaine, reporting offspring outcome up to the age of 10 (Gray et al., 2005).

In both the OPPS and MPHCD studies, cannabis use during pregnancy was not associated with increased miscarriage rates, premature deliveries or any other complications during pregnancy. Furthermore, both studies failed to link cannabis use during pregnancy with any major physical abnormalities at birth (Fried, 2002). However, in the neonatal period, the OPPS and MHPCD studies showed an association between aspects of nervous system functioning and prenatal exposure to cannabis, reflected by increased tremors that were typically accompanied by exaggerated and prolonged startles (Fried and Makin, 1987) or altered sleep patterns (Day et al., 1992).

Concerning the effects of prenatal cannabis exposure on cognitive functions in the offspring, the OPPS study found no association between prenatal cannabis exposure and infant mental development at 1 year of age (Fried and Watkinson, 1988). The high-risk MHPCD cohort, however, showed an association between the use of 1 or more joints per day during the third trimester of pregnancy and a decrease in mental scores of the Bayley Scales of Infant Development (Bayley, 1969) at 9 months of age, which disappeared at 18 months (Richardson et al., 1995).

As the children get older, there is a considerable degree of concordance in the findings of both OPPS and MHPCD cohorts. The OPPS study reported that, at 48 months, maternal cannabis use during pregnancy was significantly associated with lower scores in both the verbal and memory domains (Fried and Watkinson, 1990). Similarly, at 3-year follow up, the MHPCD study reported impairments in short-term memory and verbal and abstract/visual reasoning in children exposed in utero to cannabis (Day et al., 1994). Unlike observations made at 48 months, maternal cannabis use was not associated with deficits in global cognitive skills in 5- and 6-year-old children from the OPPS study (Fried et al., 1992a). However, at 6 years of age, prenatal cannabis exposure was associated with increased omission errors on a vigilance task, possibly reflecting a deficit in sustained attention (Fried et al., 1992b). Similar results were found in the MHPCD cohort (Leech et al., 1999). These findings lead to the hypothesis that cannabis use during pregnancy may have selective, deleterious effects on executive functions, i.e. certain higher cognitive abilities which cannot be assessed with global, standardized tests of cognition, such as the ones used at the 5- and 6-year follow ups (Fried et al., 1992a). Executive functions comprise capacities such as cognitive flexibility, sustained and focused attention, and working memory. Specific tests requiring executive functions were performed less by 9- and 12-year-old children from the OPPS cohort prenatally exposed to cannabis (Fried et al., 1998). This is consistent with findings from the MHPCD cohort, that showed problems in abstract and visual reasoning in children prenatally exposed to cannabis (Richardson et al., 2002). The deficits in executive functions induced by prenatal cannabis exposure seem to be long-lasting, since 18–22 year-old young adults from the OPPS study showed altered neuronal functioning during visuospatial working memory processing (Smith et al., 2006).

At the moment, the longitudinal OPPS and MHPCD studies are the two major sources of information about the long-term consequences of prenatal exposure to cannabis, and the considerable overlap in the findings between these two cohorts, despite the marked difference in their demographic background, is noteworthy. Overall, both these clinical studies highlight how prenatal cannabis exposure may affect high-order cognitive processes, leading to attention deficits and impairments in problem-solving tasks that require complex visuoperceptual integration. However, despite the consistency in the findings from the OPPS and MHPCD studies, it cannot be excluded that genetic and environmental variables also contribute to the relationship between maternal cannabis use and long-term neurobehavioral deficits in the offspring.

3.3. Animal studies

Because they lack some of the intrinsic limitations of epidemiological reports, animal studies examining the effects of maternal exposure to cannabinoid drugs on subsequent neurodevelopment of the offspring are of unquestionable value. Many studies carried out in laboratory animals have demonstrated that maternal exposure to high doses of cannabinoid compounds results in several morphological and functional abnormalities in the offspring (for a review on the teratogenic effects of cannabinoids see Dalterio, 1986). However, the long-term sequelae of in utero exposure to moderate doses of cannabinoid drugs have not been clearly identified yet.

More than a decade ago, Navarro et al. (1995) reviewed the behavioral consequences of maternal exposure to cannabinoids in rat models, and reported that it resulted in an alteration in the pattern of ontogeny of spontaneous locomotor and exploratory behavior in the offspring. Adult animals exposed to cannabinoid drugs during gestation and lactation indeed showed persistent alterations in the behavioral response to novelty, social interactions, sexual orientation and sexual behavior. Moreover, the offspring appeared to be sensitized to the reinforcing effects of morphine, suggesting an increased vulnerability to addictive behavior (Navarro et al., 1995).

Recently, the relation between antenatal exposure to cannabinoid drugs and cognitive outcomes in the offspring has received a great deal of attention. Prenatal exposure to a moderate dose of the CB_1 cannabinoid receptor agonist WIN55,212-2 induced a disruption of memory retention in 40- and 80-day-old rat offspring tested in the inhibitory avoidance task. Hyperactive behavior at the ages of 12 and 40 days was also found (Mereu et al., 2003). The memory impairment was associated with alterations in both hippocampal long-term potentiation and glutamate release. The decrease in hippocampal glutamate overflow was suggested to be the cause of long-term potentiation disruption, which could underlie, in turn, the long-lasting impairment of cognitive functions caused by the gestational exposure to the cannabinoid receptor agonist (Mereu et al., 2003). These findings showed that even moderate doses of cannabinoid compounds, administered during pregnancy, can have deleterious effects in the offspring, and may provide an explanation for the cognitive deficits observed in children born from mothers who smoked cannabis during pregnancy.

The same protocol of prenatal WIN55,212-2 exposure has been associated with a profound impairment in the acquisition of homing behavior, which is a simple form of learning during the early phases of postnatal life (Antonelli et al., 2005). The homing behavior test exploits the strong tendency of the immature neonate to maintain body contact with its mother and siblings. This behavior requires intact sensory, olfactory, motor and ultrasonic capabilities, as well as adequate associative and discriminative capabilities that allow the pup to become imprinted by the mother's odour, and to memorize and recognize it (Bignami, 1996). In line with the altered acquisition of homing behavior, prenatal WIN55,212-2 exposure induced a decrease in the rate of separation-induced ultrasonic vocalizations in 10-day-old rat pups (Antonelli et al., 2005). Ultrasonic emissions in rodents serve communicative purposes, since they are a potent stimulus for maternal retrieval and elicit caregiving behaviors in the dam. Since alterations in rat pup ultrasonic calling influence maternal behavior which, in turn, might affect the behavior of the offspring, the changes in ultrasonic vocalization elicited by prenatal exposure to WIN55,212-2 could have a role in the development of behavioral abnormalities later in life.

Recently, we have shown that THC, the active ingredient of cannabis, administered during the perinatal period at a dose that is not associated with gross malformations and/or overt signs of toxicity, induces cognitive impairments in the adult offspring (Campolongo et al., 2007). This study showed that the effects of moderate exposure to cannabinoid drugs on the cognitive abilities of the offspring constitute not only a long-term memory impairment, as revealed by the inhibitory avoidance test, but also a disruption in short-term olfactory memory, as assessed in the social discrimination test.

It has been shown that perinatal cannabis exposure alters morphine self-administration in the adult offspring (Gonzalez et al.,

2003; Vela et al., 1998). More recently, Spano and colleagues found that adult rats prenatally exposed to THC did not show enhanced heroin intake under normal conditions. However, enkephalin expression in brain regions implicated in reward and stress, such as the nucleus accumbens and amygdala, was altered following prenatal THC exposure. THC-exposed adult offspring showed enhanced heroin-seeking during mild stress and extinction (Spano et al., 2007). These findings suggest an altered behavioral response to stress which intensifies the motivation for drug use in THC-exposed subjects, rather than just altered sensitivity to the drug's reinforcing effects.

The possibility that in utero THC exposure induces sensitization to opiates has also been addressed by evaluating morphine- (Rubio et al., 1995) or heroin- (Singh et al., 2006) induced place conditioning in the adult rat offspring. In both cases, the results showed that THC-exposed adult offspring exhibited an enhanced sensitivity to the rewarding effects of opioid drugs.

4. Behavioral consequences of cannabinoid exposure during adolescence

4.1. Human studies

In many western countries, cannabis belongs to the most widely used illicit drugs among adolescents, leading to a variety of medical and social concerns (NIDA, 2005). Moreover, in recent years, the age of initiation of cannabis use has decreased. The number of adolescents receiving treatment at publicly funded treatment centers for cannabis abuse or dependence doubled from 1992 to 2000, and the majority of all adolescent substance abuse admissions report cannabis as the primary abused drug (Kamon et al., 2005).

The possible causal relation between cannabis use, particularly during adolescence, and psychotic and affective illness later in life has been widely investigated. It has been suggested that cannabis use by young people may be a risk factor for the occurrence of schizophrenia (Stefanis et al., 2004; Arseneault et al., 2002; Moore et al., 2007). Strong support for this association comes from a Swedish cohort study which found that heavy cannabis use at age 18 increased the risk of later schizophrenia by six (Andreasson et al., 1987). This study could not establish whether cannabis use was a consequence of pre-existing psychotic symptoms or the actual cause of psychosis. Another longitudinal prospective study, however, reported that cannabis use is associated with an increased risk of schizophrenic symptoms, even after psychotic symptoms preceding the onset of cannabis use are controlled for, indicating that cannabis use is not secondary to pre-existing psychosis (Arseneault et al., 2002). In addition, this study suggested that early cannabis use (by age 15) confers greater risk for psychosis than later cannabis use (by age 18). The strong association between early cannabis exposure and subsequent schizophrenic symptoms was confirmed by Stefanis et al. (2004). They found that both positive and negative psychotic symptoms were more strongly associated with first cannabis use below age 16 than with first use after age 15, independent of lifetime frequency of use. The association between cannabis and psychosis was not influenced by the distress associated with the experiences, suggesting that self-medication is an unlikely explanation for the association between cannabis use and psychosis. Furthermore, although cannabis use was associated with depression, the association disappeared after controlling for negative symptoms, whereas the reverse did not hold. This may explain the fact that cannabis, in a previous population study, was shown to be associated with symptoms of depression (Patton et al., 2002) that overlap with the negative symptoms of psychosis (Kibel et al., 1993).

Although other authors have found no strong evidence that cannabis use by young people induces deleterious mental health outcomes (Macleod et al., 2007, 2004), the epidemiological data discussed above suggest that heavy cannabis use by young people increases the risks of psychotic symptoms later in life. Since only a small minority of cannabis users develops psychoses, environmental factors and genetic predisposition may play an important role in the causal relation between cannabis use and psychotic illness.

Henquet and colleagues studied a large population-based sample of adolescents and young adults, and investigated prospectively whether cannabis use at baseline increases the risk of subsequent development of psychotic symptoms, whether any such increase in risk is higher in individuals with a predisposition for psychosis, and whether baseline expression predisposition increases the risk for subsequent use of cannabis. They found that cannabis use in young people moderately increased the risk of developing psychotic symptoms. The risk for the onset of symptoms was much higher in young people with a predisposition for psychosis. However, predisposition psychosis at baseline did not predict cannabis use at follow up, refuting a self-medication hypothesis (Henquet et al., 2005).

Caspi and colleagues hypothesized that the vulnerability to the deleterious effects of cannabis use by adolescents might have a genetic basis. Due to a functional polymorphism that involves a Val-to-Met substitution at codon 158, the catechol-*O*-methyltransferase (COMT) gene has two allelic variants that influence the breakdown of dopamine in the synapses. The authors of this study found that carriers of the COMT valine158 allele were most likely to exhibit psychotic symptoms and to develop schizophrenic disorders if they used cannabis. Cannabis use had no such adverse influence on individuals with two copies of the methionine allele. Evidence linking the COMT valine158 allele to schizophrenia has until now been inconsistent, and Caspi and colleagues did not observe any direct association between the Val-Met functional polymorphism and psychosis outcomes. Rather, they suggest that allelic variants of the COMT gene confer risk only to individuals additionally exposed to environmental risks, such as adolescent cannabis use (Caspi et al., 2005).

Concerning the effects of adolescent cannabis exposure on cognitive functions, young adults between the ages of 17 and 23 who smoked more than 5 joints per day had a general intelligence quotient (IQ) of 4 points lower than when they did not smoke, after accounting for confounding factors and acute intoxication (Fried et al., 2002, 2005). This decrease was not observed in past users, suggesting that the effect was reversible. One study that attempted to examine cannabis effects on adolescent cognitive neurodevelopment differentiated long-term heavy users into early users before the age of 17 and late users, and compared them to a minimal cannabis exposed control group (Pope et al., 2003). Here, differences in verbal IQ were found following 28 days of abstinence. Although this may indicate a neurotoxic effect of cannabis, the authors recognized that pre-existing differences or underlying factors predisposing to both impaired cognition and early cannabis use may explain the observation. However, in line with this finding, Ehrenreich et al. (1999) reported that adults who smoked cannabis before, but not after, the age of 16 had poorer performance in a task that required focused attention. Together, these findings suggest that the effects of cannabis on cognition might depend on the age of initiation of cannabis use.

Significant social concerns surround cannabis as an entry point to other drugs of abuse. Two main theories have been proposed to explain the relationship between the use of cannabis and other illicit drugs. The gateway theory argues that early cannabis exposure alters brain reward pathways, thus facilitating the subsequent use of other drugs. In support of this theory, Fergusson et al. (2006, 2002) demonstrated that regular or heavy cannabis use by the ages of 14 or 15 was strongly associated with other illicit drug use, even after family and social circumstances were controlled for. A similar effect for nicotine was also recently found, as early (14–15 years old) but even infrequent cannabis use increased the risk for subsequent nicotine dependence (Patton et al., 2005). Although these studies are consistent with a neurodevelopmental effect of cannabis on reward pathways, genetic or environmental factors cannot be excluded. The

correlated vulnerabilities theory argues that cannabis use and other illicit drug use are associated because both drugs are influenced by a single common liability; in other words, some individuals may have a general predisposition to using drugs, including cannabis, possibly due to a risk-taking personality (Morral et al., 2002).

In recent years, the relative validity of the gateway and common liability models has been the topic of intense debate. Information from twin pairs can be helpful in examining these models. In particular, the discordant twin design can compare the risk of subsequent other illicit drug use in twin pairs discordant for cannabis use. Lynskey et al. (2003) analyzed this relationship and found that, compared to their non-user co-twins, twins with early cannabis use had a 2.6–5.2 times higher risk of using other illicit drugs, thus suggesting that genetic factors were not of major importance. Conversely, however, another twin study found evidence to support genetic factors in mediating early cannabis use and later substance use (Agrawal et al., 2004). Therefore, human studies are inconclusive as to whether cannabis use has a direct causal influence on other illicit drug use, whether the two are related by a common liability, or if the association results from a combination of correlated and causal processes.

4.2. Animal studies

Despite the increasing use of cannabis among adolescents, there is scarce information about the effects of cannabinoid drugs in adolescent experimental animals. In rodents, cannabinoids induce a characteristic tetrad of behavioral effects: hypolocomotion, antinociception, hypothermia and catalepsy (Compton et al., 1993; Martin et al., 1991). Wiley et al. (2007) found that the overall pattern of acute THC-induced tetrad effects was similar in adolescent and adult rats of both sexes. However, male adolescents were less sensitive to the hypolocomotor and hypothermic effects of THC after repeated administration. In line with this finding, Schramm-Sapyta et al. (2007) showed that acute THC has less aversive and locomotor-reducing effects in adolescent than adult rats. Similarly, Quinn et al. (2007) reported that adolescent rats find repeated THC exposure less aversive than adults in a place conditioning paradigm. Together, these findings suggest that certain characteristics of the immature adolescent brain may make adolescent rats less sensitive to the use-limiting aversive properties of THC. Translating these results to the human situation, an overall reduced sensitivity to the undesirable effects of THC in adolescents may contribute to the frequent and continued pattern of cannabis use which characterize this age.

Adolescent rats may, however, be more susceptible than adults to some effects of chronic THC exposure. The study by Quinn et al. (2007) showed that adolescent rats display greater cognitive deficits than adult rats following repeated THC exposure. Indeed, the notion that cannabinoid drugs induce more severe cognitive effects in adolescent than in adult rats is supported by several other studies. Repeated THC treatment impaired spatial learning in the Morris water maze task more in adolescent than in adult rats (Cha et al., 2007, 2006). Similarly, deficits in the object recognition task were found after chronic treatment with the CB_1 cannabinoid receptor agonist CP 55,940 in adolescent but not adult rats (O'Shea et al., 2004). Schneider and Koch (2003) showed that chronic pubertal treatment with WIN55,212-2 resulted in an impaired object recognition memory in adulthood. Furthermore, pre-pubertal treated rats showed a disrupted prepulse inhibition of the acoustic startle response and lower break points in a progressive ratio operant behavioral task (Schneider and Koch, 2003). Since prepulse inhibition deficits, object recognition memory impairments, and anhedonia/avolition are among the endophenotypes of schizophrenia, the authors of this study proposed chronic cannabinoid administration during pubertal development as a neurodevelopmental animal model for some aspects of schizophrenia (Schneider and Koch, 2003). Again, it is worth noting that if chronic cannabinoid treatment was administered in adulthood, none of the tested behaviors was affected (Schneider and Koch, 2003).

Recently, Ellgren et al. (2007) evaluated whether adolescent exposure to THC alters opiate intake and limbic opioid innervation in adulthood. They found that adult animals exposed to THC during adolescence responded more for heroin at moderate to low doses and had higher heroin intake during the drug maintenance phase. Furthermore, THC-exposed animals showed changes of the endogenous opioid system in brain regions involved in reward processes, such as the nucleus accumbens shell, ventral tegmental area and substantia nigra. The authors interpret their findings as support for the controversial gateway hypothesis that adolescent cannabis exposure has a long-lasting impact on hedonic processing, resulting in enhanced heroin intake. It should be noted, however, that THC exposure during adolescence did not appear to predispose animals to an increased sensitivity to initiate heroin self-administration, since both vehicle- and THC-exposed rats reached stable heroin self-administration behavior between day 6 and 7 of the acquisition phase. Furthermore, it is also important to note that the evidence for the gateway hypothesis using animal models of drug addiction (Rubio et al., 1995; Singh et al., 2006; Spano et al., 2007) does not exclude the contribution of other factors such as genetics, environment, and social issues that could influence the direct neurobiological effects of early THC exposure to either enhance or attenuate the progression to adult drug abuse. Caution is therefore advised when extending the results of these studies to human drug abuse.

The effects of cannabinoid drugs on the emotional reactivity of adolescent subjects are inconsistent and, sometimes, sex-dependent. Exposure to CP 55,940 during adolescence was associated with increased anxiety, as CP 55,940-treated male (O'Shea et al., 2006) and female (O'Shea et al., 2004) rats showed a significant decrease in social interactions with conspecifics. If animals were chronically treated during adulthood, male (O'Shea et al., 2006) but not female (O'Shea et al., 2004) rats showed reduced social interaction, thus suggesting that adult males are more sensitive than adult females to the detrimental effects of chronic cannabinoid exposure.

Chronic treatment with CP 55,940 during adolescence, however, has also been reported to increase the percentage of time spent on the open arms of the elevated plus-maze in adulthood, indicative of an anxiolytic effect (Biscaia et al., 2003). The effects of CP 55,940 in the elevated plus-maze were more pronounced in female than in male rats. However, in this study, control females showed higher locomotor activity and decreased levels of emotionality than the control males. For this reason, it is difficult to conclude whether this differential effect may reflect either a sex-specific response to chronic CP 55,940 treatment, or a more general sex-specific difference in the response in this task. In contrast with this finding, acute THC exposure induced anxiogenic-like effects in the elevated plus-maze and light–dark box tests in both adolescent and adult rats. The drug, however, was more anxiogenic in adult than adolescent rats (Schramm-Sapyta et al., 2007). Although this result seems to confirm that adolescent rats are less susceptible to some of the adverse effects of cannabinoid exposure, the greater activity of adolescents in the elevated plus-maze and their faster emergence into the light during the light–dark test indicate that, compared to adults, adolescent rats may be less behaviorally inhibited in these conflict-associated tasks. Again, it is therefore difficult to conclude whether the different effects of THC in adolescent and adult rats reflect an age-specific response to THC, or more general age-related behavioral differences.

Recently, we have shown that cannabinoid neurotransmission plays an important role in the modulation of social play behavior in adolescent rats, with opposite behavioral outcomes depending on how the endocannabinoid system is stimulated (Trezza and Vanderschuren, 2007). The CB_1 cannabinoid receptor agonist WIN55,212-2 reduced social play behavior. In contrast, the indirect cannabinoid agonist URB597, which increases endocannabinoid signalling by inhibiting fatty acid amide hydrolase (FAAH), the enzyme that catabolises the endocannabinoid anandamide, enhanced social play.

This effect of URB597 depended on opioid and dopaminergic neurotransmission, because it was blocked by the opioid receptor antagonist naloxone and the dopamine receptor antagonist alpha-flupenthixol. Interestingly, the play-enhancing effect of morphine was reduced by the CB_1 cannabinoid receptor antagonist SR141716, but not by alpha-flupenthixol. Moreover, combined treatment with ineffective doses of morphine and URB597 enhanced social play. Together, this suggests that endocannabinoid and opioid systems jointly facilitate social play in adolescent rats, but through dissociable mechanisms. These results can have scientific, social and clinical relevance. First, social play behavior, the first form of non-mother directed social behaviors displayed by most adolescent mammals, is essential for social, cognitive and sexual development (Vanderschuren et al., 1997), and similarly important for human children (Ginsburg, 2006). Second, the social consequences of cannabinoid exposure during adolescence have been, so far, quite controversial, as both prosocial (Goode, 1970) and antisocial (D'Souza, 2007) effects have been reported following cannabis exposure in humans. We have solved this paradox, by showing that enhancing endocannabinoid tone within the neural circuits mediating social behavior facilitates sociability, but stimulating cannabinoid neurotransmission outside this circuitry may attenuate the ability to execute complex social acts. Third, although the potential abuse liability of URB597 deserves further investigation, our results broaden the potential therapeutic utility (Piomelli et al., 2006) of this indirect agonist and support a role for anandamide in psychopathological disorders accompanied by disturbances of social interactions, such as autism. Last, our results demonstrate that the neuronal mechanisms underlying functional cannabinoid–opioid interactions are already mature in adolescent animals.

5. Conclusions

Extensive research carried out during the past two decades has demonstrated the existence of an endocannabinoid system in the CNS and also in the periphery, which consists of G-protein-coupled receptors and endogenous ligands. This system, which is the target of psychoactive cannabis compounds, is thought to have modulatory actions in several neurobiological processes, as has been proposed from the anatomical distribution of cannabinoid receptors in the brain and from the well-known pharmacological effects of cannabinoid-related compounds.

The endocannabinoid system is present in the CNS since early stages of brain development, and it plays a relevant role in brain organization during pre- and postnatal life (Fernandez-Ruiz et al., 2000; Fride, 2004). Several studies have described the presence of CB_1 cannabinoid receptors (Rodriguez de Fonseca et al., 1993) and their endogenous ligands, anandamide and 2-AG, in the developing brain (Berrendero et al., 1999). The atypical, transient localization of CB_1 cannabinoid receptors during the perinatal period suggests a specific involvement of the endocannabinoid system in brain development. Moreover, the presence of CB_1 cannabinoid receptors during brain development has been associated with neuroprotective effects in the maturation of the CNS and its functions (Fernandez-Ruiz et al., 2000; Fride, 2004). CB_1 cannabinoid receptor density (Rodriguez de Fonseca et al., 1993) and mRNA levels (McLaughlin and Abood, 1993) progressively increase during postnatal development, peaking between PND 30 and 40, shortly before the onset of puberty. CB_1 cannabinoid receptor levels decrease afterwards until reaching adult values (Rodriguez de Fonseca et al., 1993).

Developmental studies on the effects of cannabinoid drugs are of special relevance for several reasons. First, cannabis preparations are the illicit drugs most widely used by pregnant women in Western countries (Fried, 2002). Since the psychoactive ingredients of cannabis can cross the placenta and be secreted in the maternal milk (Hutchings et al., 1989; Jakubovic et al., 1977), cannabis use and abuse during pregnancy and lactation may have long-lasting neurobehavioral effects on the offspring. Second, cannabis use has increased in adolescents, whose developing brain might be particularly susceptible to social and environmental influences. Thus, cannabis exposure at this critical developmental age may lead to neurobehavioral alterations or induce neuropsychiatric disorders, such as schizophrenia, later in life. Third, the endocannabinoid system has been proposed as a novel therapeutic target for the treatment of some neuropsychiatric diseases (Piomelli et al., 2006; Vinod and Hungund, 2006), including neurodevelopmental disorders, such as ADHD. However, the potential therapeutic application of cannabinoid drugs in children requires a better knowledge of the effects of these compounds on the CNS of immature individuals.

The studies reviewed here suggest that changes in the activity of the endocannabinoid system during stages of high neuronal plasticity, such as the perinatal and adolescent period, can have long-lasting behavioral consequences. Epidemiological studies present several methodological limits which make it difficult to strictly control all the factors that potentially influence the relationship between cannabis exposure at these important developmental ages and subsequent neurobehavioral outcomes. Conversely, although animal models allow the strict monitoring of the influence of confounding factors usually present in human studies, they do not take into account environmental and social issues that could influence the neurobiological effects of early cannabis exposure. Thus, further investigation and combined preclinical and clinical approaches are needed before a causal relationship between developmental cannabis exposure and long-lasting neurobehavioral outcomes can be firmly established.

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