

Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure

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ABSTRACT

During puberty, neuronal maturation of the brain, which began during perinatal development, is completed such that the behavioral potential of the adult organism can be fully achieved. These maturational events and processes of reorganization are needed for the occurrence of adult behavioral performance but simultaneously render the organism highly susceptible to perturbations, such as exposure to psychoactive drugs, during this critical developmental time span. Considering the variety of maturational processes occurring in the endocannabinoid system during this critical period, it is not surprising that the still-developing brain might be highly susceptible to cannabis exposure. Emerging evidence from human studies and animal research demonstrates that an early onset of cannabis consumption might have lasting consequences on cognition, might increase the risk for neuropsychiatric disorders, promote further illegal drug intake and increase the likelihood of cannabis dependence. These findings suggest that young people represent a highly vulnerable cannabis consumer group and that they run a higher risk than adult consumers of suffering from adverse consequences from cannabinoid exposure. The aim of the present review is to provide an overview over the possible deleterious residual cannabinoid effects during critical periods of postnatal maturation and to offer a more precise delineation of the vulnerable time window for cannabinoid exposure.

Keywords Adolescence, cannabinoids, cognitive effects, drug addiction, endocannabinoid maturation, pubertal timing.

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INTRODUCTION

It is widely accepted that the subjective and neurobiological consequences of cannabis use depend to a great extent upon the dosage and history of its use. In addition, the age of onset of cannabis consumption is clearly an important factor for the acute and later consequences of cannabis consumption, the recognition of which has gained increasing attention. The use of cannabis has long been considered relatively harmless. However, recently concern has been growing about possible adverse health effects, which appear to be most pronounced among those who start using cannabis at a young age, mainly during mid-adolescence or puberty (e.g. Ehrenreich *et al.* 1999; Pope *et al.* 2003; Arseneault *et al.* 2004; Hall 2006b,c). The adolescent years thus seem to be a crucial period for the development of cannabis-related harm. Although the association between early cannabis use and subsequent problems may be due in part to common risk factors, it nevertheless remains important to monitor the

age of initial cannabis use. It has been shown in the last decades that the use of cannabis did increase in young people and the age of first use has declined with most consumers starting cannabis use in their mid to late teens (Monshouwer *et al.* 2005; EMCDDA 2006; Hall 2006b). Therefore, those who might be at the highest risk for adverse consequences of cannabis exposure tragically represent the major consumer group of cannabis derivatives.

Human studies, in particular those using retrospective evaluations, have some limitations because of the vast heterogeneity of cannabis consumption. Hence, research with laboratory animals offers an important link to gaining further knowledge about specific effects of cannabinoids during postnatal development and underlying neurobiological mechanisms mediating this heightened susceptibility. The present review will therefore survey the possible deleterious cannabinoid effects during critical periods of adolescent/pubertal maturation reported in both human studies and animal research. A further

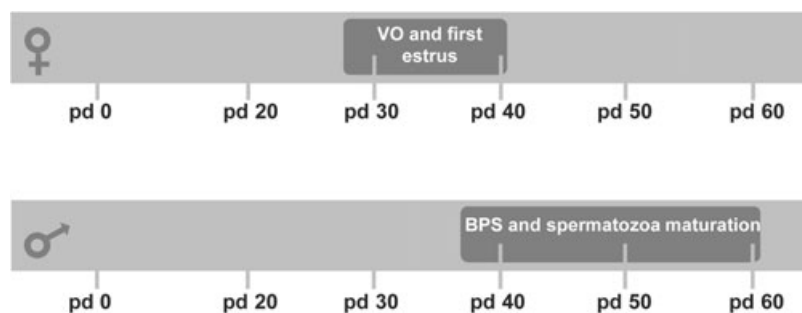


Figure 1 Timing of pubertal maturation in female and male rats. The pubertal period in female rats (approximately postnatal day (pd) 28 to pd 40) is determined by vaginal opening (VO) and first estrus. Balano preputial separation (BPS) indicates pubertal onset in male rats (around pd 40), and sexual maturity is indicated by the presence of mature spermatozoa in the vas deferens, which is achieved around pd 60

aim will be to more precisely delineate the vulnerable time window for cannabinoid exposure during postnatal developmental periods.

NEUROBEHAVIORAL CHARACTERISTICS AND TIMING OF PUBERTY AND ADOLESCENCE

Puberty and adolescence are important developmental periods during which a child matures into an adult. The term 'puberty' (lat. *pubertas* = (sexual) maturity), which has to be clearly distinguished from 'adolescence' (lat. *adolescere* = to grow up), refers exactly to the time period during which sexual maturation is achieved and is initiated by an increased secretion of gonadotropin-releasing hormone (GnRH), resulting in gonadal maturation and steroid hormone secretion. Although puberty and adolescence are overlapping time periods, with puberty being a part of adolescence, the terms cannot be used interchangeably. Adolescence refers to the gradual period of behavioral transition from childhood to adulthood and the boundaries of this period are less precisely defined (for review see Spear 2000; Sisk & Foster 2004). However, gonadal alterations in puberty and adolescent behavioral maturations are intimately linked in timing through multiple and complex interactions between the nervous system and gonadal steroid hormones, which are involved in the maturation of reproductive behavior (e.g. sexual salience of sensory stimuli and sexual motivation) (Sisk & Foster 2004). During puberty, major differences of size, shape, composition and function develop in many body structures and systems. Numerous neurodevelopmental alterations take place during this period, such as maturational processes in the medial prefrontal cortex (mPFC) and limbic regions, which are characterized by both progressive and regressive changes, e.g. myelination and synaptic pruning (Spear 2000; De Bellis *et al.* 2001; Powell 2006). Furthermore, maturation of neurotransmitter systems such as the glutamatergic, the dopaminergic and also the endogenous cannabinoid system occur

during adolescence, with developmental peaks often seen concomitant with the onset of puberty (Rodriguez de Fonseca *et al.* 1993; Andersen *et al.* 2000; Spear 2000).

Because the exact timing of adolescence is rather difficult to define in humans, an exact definition of this period in laboratory animals is even more complicated and opinions differ about the precise time window that should be defined as adolescence (for review see Spear 2000). However, clarification of this issue is of great importance if animal research is expected to give indications about critical time windows for cannabinoid exposure. In her excellent review, Spear highlighted the time window from postnatal day (pd) 28–42 as a prototypic adolescent period during which animals of most breeding stocks would exhibit adolescent-typical characteristics. However, she also points out that this conservative age range does not imply that older or younger animals could no longer be considered as adolescents. This is true in particular for male animals whose neurobehavioral and physical pubertal maturation reaches beyond the suggested time window.

In contrast to adolescence, timing of puberty is a lot easier to determine in laboratory rats, because external physical signs exist for both female [vaginal opening (VO)] and male rats [balano preputial separation (BPS)], indicating that the onset of this specific developmental period has occurred. In male rats, BPS normally occurs around pd 40 (Korenbrodt, Huhtaniemi & Weiner 1977; Fernandez-Fernandez *et al.* 2005), and in the female rat VO is observable around pd 35 in most laboratory stocks (e.g. Engelbregt *et al.* 2001; Delemarre-van de Waal *et al.* 2002; Fig. 1). Ojeda and Urbanski have suggested a juvenile period for the male rat reaching from pd 22 to pd 35, followed by a peripubertal period from pd 36 (shortly before the physiological onset of puberty around pd 40) until fertility is reached around pd 60, indicated by the presence of mature spermatozoa in the vas deferens (for review see Clegg 1960; Ojeda & Urbanski 1994). The latter is an important point that should be thoroughly recognized when adult controls are chosen for

developmental studies. For instance, male animals that are just reaching maturity at an age of pd 60–65—in their late adolescence—are sometimes used as an ‘adult’ control, which distorts the interpretation of these findings in comparison with data from adolescent/pubertal animals.

For female animals, a juvenile period can be defined from pd 22 until shortly before pd 30, followed by a peripubertal period with variable duration that culminates with the occurrence of first ovulation (around pd 38) (for detailed review see Ojeda & Urbanski 1994). Similar gender differences are found in humans and across other mammalian species with females normally maturing more rapidly than males (Spear 2000). Although there is a wide range of normal ages, on average, girls begin the process of puberty about 1–2 years earlier than boys and reach completion in a shorter time.

Because the most important adolescent behavioral changes and many important neuronal maturational processes are closely linked to pubertal development (Sisk & Foster 2004), the more exact time window of puberty (around pd 38 to pd 60 in males and pd 28 to ~pd 40 in females) (Fig. 1) might be the better choice for studies in the laboratory rat for the evaluation of critical developmental periods. It is also of great importance for animal research to be mindful of gender differences occurring in the timing of puberty and adolescence in female and male animals (with females maturing earlier than males), in particular for studies comparing both sexes.

POSTNATAL DEVELOPMENT OF THE ENDOCANNABINOID SYSTEM

Endocannabinoids and their cannabinoid receptors, CB1 and CB2, are present from the early stages of gestation and play a number of vital roles for the developing organism (Fernandez-Ruiz *et al.* 1999, 2000). However, only a few studies have so far investigated the maturational processes occurring in the endocannabinoid system during further postnatal development, including adolescence and puberty. A thorough study by Rodriguez de Fonseca and coworkers demonstrated a sex-dependent progressive increase in CB1 receptor radioligand binding in rats in the limbic system, mesencephalon and striatum. Binding increased gradually starting from pd 10 and reached maximum values around pd 30 in females and pd 40 in males, respectively (Rodriguez de Fonseca *et al.* 1993). Afterwards, CB1 binding seems to decrease during the pubertal period until it reaches adult values (measured on pd 70). Interestingly, the peak of CB1 receptor binding coincided in male and female animals, with the age of approximate onset of puberty. A similar postnatal increase in CB1 receptor binding was reported in other studies in rats for the striatum, cerebellum and the cortex

(Belue *et al.* 1995) as well as for the whole brain (McLaughlin *et al.* 1994). Additionally, Berrendero *et al.* (1999) detected an increase in anandamide (AEA) levels during the early postnatal period. However, in all these studies animals were only investigated up to pd 21 and again on pd 60/adulthood, omitting the pubertal developmental period. Interestingly, CB1 mRNA expression is present in the whole brain as early as pd 3 (McLaughlin *et al.* 1994) and does not differ from later postnatal (only tested up to pd 21) or adult expression levels (pd 60), as has been shown for CB1 binding. The authors suggested that the observed progressive increase in CB1 binding might reflect the final manifestation of complete functional maturity of the receptors. These interpretations were supported by a study investigating the development of behavioral responses to exogenous and endogenous cannabinoids in mice from pd 6 up to the age of weaning (pd 20) and in adulthood (Fride & Mechoulam 1996). A significant behavioral response (locomotor activity and analgesia) as seen in adult mice to either AEA or Δ^9 -tetrahydrocannabinol (THC) was not observed in juvenile mice at any postnatal age tested. Unfortunately, both studies did not include the pubertal period in their investigations.

Although developmental studies examining CB1 receptor distributions in the human brain are rare, a similar progressive increase from early prenatal stages to adulthood in different regions (e.g. hippocampus, frontal cortex, basal ganglia and cerebellum) has been reported by Mato, Olmo & Pazos (2003). In this study, autoradiographic levels of CB1 receptors were lowest in fetal/neonatal cases (week of gestation: 19 to 40), slightly increased in the brains of infants/children (3 to 96 months of age), and the highest binding was observed in adults (22 to 74 years), indicating a similar maturational pattern of the endocannabinoid system in humans as has been reported in the laboratory rat, although the pubertal period was not addressed in this study.

In addition to the progressive increase in CB1 binding during development, it has been shown that hypothalamic levels of the endocannabinoid AEA display a peak immediately before the onset of puberty in female rats, indicating a possible involvement of the endocannabinoid system in the timing of puberty (Wenger *et al.* 2002). Accordingly, chronic THC treatment delays the onset of puberty in female rats for 2 days (Wenger, Croix & Tramu 1988), and it has been shown *in vitro* that CB1 and CB2 receptors are expressed on GnRH neurons and that these neurons are able to produce and release endocannabinoids (AEA and 2-arachidonyl-glycerol) (Gammon *et al.* 2005). A close interaction between the endocannabinoid system/cannabinoids and sex steroids has been described before (e.g. Murphy, Rodriguez & Steger 1991; Rodriguez *et al.* 1994), and this interaction

might contribute to the major developmental changes occurring in the endocannabinoid system during pubertal maturation.

In summary, the activity of the endocannabinoid system, including receptors and endogenous ligands, seems to be highest around puberty onset, indicating a high vulnerability of this specific developmental period for the consequences of exogenous cannabinoids. Consistent with these findings, we could previously show that chronic treatment with the synthetic cannabinoid receptor agonist WIN 55,212-3 (WIN) throughout the pubertal period (pd 40–65) of male rats leads to more pronounced persistent behavioral alterations in adulthood than a comparable treatment in prepubertal animals (pd 15–40) (Schneider & Koch 2003; Schneider, Drews & Koch 2005), indicating the high susceptibility of the endocannabinoid system for perturbations during this critical time window of puberty. However, many questions regarding the maturation of the endocannabinoid system, in particular during the critical period of pubertal development, remain open and require further research.

SPECIFIC EFFECTS OF PUBERTAL CANNABINOID EXPOSURE

It has been suggested that puberty serves as a developmental period when the organization of the brain that began during perinatal development is completed and the behavioral potential of the adult organism can be fully achieved (Romeo 2003). These maturational processes are necessary for the occurrence of adult behavioral performance but simultaneously render the organism vulnerable to perturbations during this critical developmental time span (Chambers, Taylor & Potenza 2003). Regarding the various developmental processes occurring in the endocannabinoid and related neurotransmitter systems during puberty, it is not surprising at all that immature mammals seem to be particularly susceptible to the exposure of exogenous cannabinoids. It has been shown in humans (e.g. Ehrenreich *et al.* 1999; Wilson *et al.* 2000; Patton *et al.* 2002; Pope *et al.* 2003; Arseneault *et al.* 2004; Caspi *et al.* 2005) and animals (e.g. Schneider & Koch 2003; O'shea *et al.* 2004; Pistis *et al.* 2004; Schneider & Koch 2005, 2007; Cha *et al.* 2006) that consumption/administration of cannabinoids during pubertal development induces lasting behavioral and morphological alterations, which are absent or attenuated after an identical cannabis exposure in adulthood. The following sections will give an overview over these possible adverse consequences of cannabis exposure during periods around pubertal maturation.

Effects on cognition

It is widely accepted that cannabinoids acutely impair cognitive processes such as short-term memory function or attention (e.g. Miller & Branconnier 1983; Hall & Solowij 1998; Sullivan 2000). However, controversy remains as to the residual long-term deleterious effects of cannabis exposure on cognitive functioning. So far most studies seem to argue against this possibility (e.g. Lyketosos *et al.* 1999; Pope *et al.* 2001). However, during the last decade the age of first cannabis use has gained increasing interest. Studies that take the age of onset of cannabis consumption into consideration show a completely different picture than those that do not differentiate between early- and late-onset consumers.

Ehrenreich *et al.* (1999) found that early-onset cannabis users (before age 17), but not late-onset users (onset at age 17 or later), exhibited significantly longer reaction times than controls in a visual scanning task. Another study compared long-term cannabis users who had initiated use before age 17 with users that started to consume cannabis when they were older than 17 (Wilson *et al.* 2000). Magnetic resonance imaging revealed that, compared with late-onset users, early-onset users had a lower percentage of gray matter and a higher percentage of white matter relative to whole brain volume. Positron emission tomography evaluation in the same subjects showed that male early-onset users had higher cerebral blood flow than males initiating use after age 17. In addition, early-onset users were shorter in height and lower in weight than late-onset users. Pope *et al.* (2003) further found that first cannabis use before age 17 was associated with poorer cognitive performance, especially in verbal IQ, than late-onset users or control subjects.

Although these findings from human retrospective studies point out the risk of early cannabis use for cognitive functioning in a similar way, they also have some limitations. First, they do not inform about the pattern of initial cannabis ingestion at young ages, whether cannabis was consumed regularly or just occasionally by the young users. Furthermore, there is a high variation in abstinence periods before testing was done [Pope *et al.* (2003): 28 days; Wilson *et al.* (2000): 14 days; Ehrenreich *et al.* (1999): ~30 hours] and in criteria defining regular cannabis consumption (e.g. Pope *et al.* (2003): subjects had to smoke cannabis at least 5000 times in their lives; Ehrenreich *et al.* (1999): once per week consumption over half a year). In addition, due to the enormous heterogeneity of consumption patterns (different inhalation techniques, oral ingestion) and cannabis products (marijuana, hashish, hashish oil), the final bioavailability of THC differs extremely even between users showing a similar history of use.

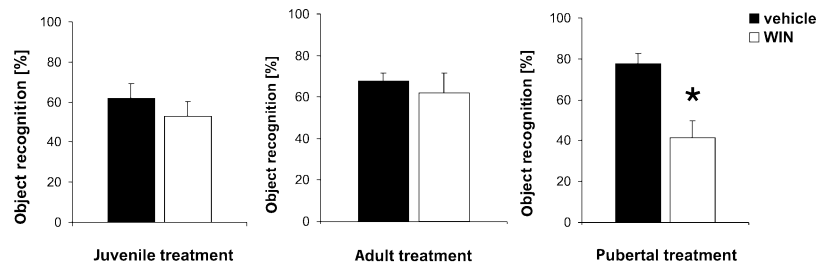


Figure 2 Residual effects on object recognition memory in rats after chronic treatment with the cannabinoid receptor agonist WIN 55,212-2 during different developmental periods. Persistent significant impairments in recognition memory performance (tested in adult animals at least 15 days after treatment cessation) were exclusively observed after chronic pubertal treatment (modified from Schneider & Koch 2003; Schneider *et al.* 2005; significant effects are indicated by asterisk)

Taking these limitations into account, animal research offers much better possibilities for a controlled investigation of the direct association between cannabinoid exposure during maturation and subsequent disturbances in cognitive processing. The first studies investigating residual effects after postnatal cannabinoid exposure were done by Stiglick and Kalant; they demonstrated that chronic exposure of immature animals to THC caused more irreversible residual effects on cognitive performance (Stiglick & Kalant 1982a,b) than chronic treatment of mature rats (Stiglick & Kalant 1985). However, treatment periods in this study were relatively long (3 to 6 months) so that it remained difficult to isolate the specific vulnerable period in detail. We have shown recently that chronic treatment with the cannabinoid receptor agonist WIN throughout the period of pubertal development in male rats (pd 40–65) leads to long-lasting behavioral disturbances in adulthood. A comparable treatment in adult (> pd 70) and prepubertal (pd 15–40) rats induces no or only minor lasting impairments on behavioral performance, respectively, identifying puberty as the highest vulnerable period for the adverse effects of exogenous cannabinoids (Schneider & Koch 2003; Schneider *et al.* 2005) (Fig. 2). Pubertal WIN-treated rats showed persistent alterations in sensorimotor gating, object recognition memory, progressive ratio performance, social behavior and wake–sleep rhythm (Schneider & Koch 2003, 2005). These findings were confirmed by two studies showing that chronic treatment with the cannabinoid receptor agonist CP 55,940 during puberty in female rats (pd 30–50) (O’Shea, McGregor & Mallet 2006) and during early puberty in males (pd 34–55) (Quinn *et al.* 2008) persistently and specifically affected object recognition memory. Furthermore, it has been shown that acute THC treatment impaired both spatial and non-spatial learning in the water maze more powerfully in male juvenile rats (pd 30) than in adults (pd 65–70), whereas no residual alterations were seen in this study after chronic

treatment from pd 30 to 50 (Cha *et al.* 2006). However, treatment in these male rats started 10 days before puberty onset and ended about 10 days before sexual maturity was reached and might therefore not have been sufficient to observe persistent effects. This is confirmed by our recent findings that chronic WIN treatment throughout the juvenile period (pd 15–40) did not induce residual alterations in working memory when tested in adulthood (Schneider *et al.* 2005) (Fig. 2). It has been shown that juvenile male rats show an attenuated behavioral response to catecholamine agonists between pd 30 and pd 40 compared with younger or older animals, and this hyposensitivity might be related to the functional maturation of dopamine autoreceptors in mesolimbic brain regions during this period, which further results in a temporary decrease in the activity of mesolimbic dopamine projections (Spear & Brake 1983). Because close interactions between the endogenous cannabinoid system and the dopaminergic system are well established (e.g. Giuffrida *et al.* 1999; van der Stelt & Di Marzo 2003; Gardner 2005), cannabinoids might generate fewer or different effects during the period just before puberty onset. Interestingly, the same group reporting adolescent-specific residual cannabinoid effects on object recognition (O’Shea *et al.* 2004; Quinn *et al.* 2008) failed in an additional study to confirm these findings (O’Shea *et al.* 2006). That study even reported similar residual alterations in object recognition memory, irrespective of age when chronic treatment occurred (pd 4–24, pd 30–50 and pd 56–76), and it is the only study showing persistent effects after adult cannabinoid exposure. However, this alleged ‘adult’ treatment was started in immature males at an age of 56 days before sexual maturity was reached (Clegg 1960).

Taken together, these findings from animal studies confirm the previous results from human studies and indicate puberty/mid-adolescence as a highly susceptible time window for possible residual (and also acute) effects on cognitive processing.

Implications for neuropsychiatric disorders

Global evidence indicates that cannabis use acts as a modest statistical risk factor for the emergence of psychosis, ranging from psychotic symptoms such as hallucinations and delusions to clinically significant disorders such as schizophrenia (Caspi *et al.* 2005). There is little dispute that cannabis intoxication can lead to acute transient psychotic episodes in some individuals (D'Souza *et al.* 2000; Skosnik, Spatz-Glenn & Park 2001) and that it can produce short-term exacerbation, recurrences as well as an earlier onset of psychotic symptoms (Hall & Degenhardt 2000; Jockers-Scherubl *et al.* 2003; Veen *et al.* 2004). However, controversy remains about whether cannabis use can actually cause schizophrenia in the long term. Prospective studies could show that using cannabis in adolescence increases the likelihood of experiencing symptoms of schizophrenia in adulthood. Cannabis use has been associated with an increased risk of experiencing schizophrenic symptoms, even after psychotic symptoms preceding the onset of cannabis use are controlled for, indicating that cannabis use is not secondary to a pre-existing psychosis. Furthermore, early cannabis use (by age 15) entails a greater risk for schizophrenia outcomes than later cannabis use (by age 18) (for detailed review see Arseneault *et al.* 2004), indicating again the high susceptibility of the pubertal developmental period for the deleterious effects of cannabinoids.

Although the majority of young people are able to use cannabis in adolescence without harm, a vulnerable minority experience harmful outcomes. Therefore, if cannabis is indeed causal, some individuals may be genetically vulnerable to its effects. This hypothesis has been tested by Caspi and colleagues in a longitudinal study of a representative birth cohort followed to adulthood (Caspi *et al.* 2005). A functional polymorphism in the catechol-O-methyltransferase (COMT) gene was found to have moderated the influence of adolescent cannabis use on developing adult psychosis. Carriers of the COMT valine158 allele were most likely to exhibit psychotic symptoms and to develop schizophreniform disorders if they used cannabis during adolescence. Cannabis use had no such adverse influence on individuals with two copies of the methionine allele. The results of this study provide evidence that adolescent cannabis use, but not adult-onset use, is associated with later psychosis outcomes. This suggests that the observed gene-environment interaction may be limited to a sensitive period of brain development in adolescence.

Similar indications were observed in animal studies, where chronic pubertal, but not adult, cannabinoid treatment resulted in lasting behavioral deficits, resembling at least some aspects of schizophrenic symptoms.

Cannabinoid exposure during pubertal development induced working memory deficits (Schneider & Koch 2003; O'Shea *et al.* 2004; Quinn *et al.* 2008), impaired sensorimotor gating, and led to abnormal social behavior and anhedonia in adulthood (Schneider & Koch 2003, 2005). Some of these behavioral deficits were even more pronounced if the animals were rendered more vulnerable to the effects of the pubertal cannabinoid administration by neonatal lesion of the mPFC on pd 7 (Schneider & Koch 2005, 2007), indicating that susceptible individuals show a higher risk for adverse consequences after cannabis exposure.

There is also increasing evidence that regular, and in particular heavy cannabis use, might be linked to depression, anxiety and other mood-related disorders in a gender-specific way (e.g. Patton *et al.* 2002; Rey *et al.* 2002; Degenhardt, Hall & Lynskey 2003; Poulin *et al.* 2005). Similar to the risk for schizophrenia, this association has been reported to be mainly linked to an early onset of problematic cannabis use in young people (Degenhardt *et al.* 2003). Data from animal studies on emotional behavior and anxiety are partially conflictive, depending strongly on the age when cannabinoid exposure took place. In pubertal rats (pd 40), CP 55,940 induced hyperreactivity and the rats emitted audible vocalizations when picked up by the experimenter, which might be interpreted as an aversive or anxiogenic-like response (Romero *et al.* 2002). In addition, we have found in a previous study that chronic treatment with the cannabinoid agonist WIN in pubertal (pd 40–65) rats after neonatal mPFC lesion alters the pattern of social play behavior in adult animals in a way that could be interpreted as increased anxiety (Schneider & Koch 2005). Additionally, chronic juvenile (pd 15–40) and pubertal WIN treatment reduced the time spent in the center of an open field and the number of rearings in adulthood, indicating reduced exploratory and increased anxiety-related behavior (Schneider & Koch 2005; Schneider *et al.* 2005). Consistent with these findings, it was shown that chronic cannabinoid treatment during puberty in female rats (pd 30–50) resulted in persistent alterations in the social interaction test, indicating increased anxiety (O'Shea *et al.* 2004). However, a more recent study by the same group failed to show an adolescent exposure-specific effect of THC on social interaction, because social interaction was affected in all animals irrespective of the age when treatment took place (O'Shea *et al.* 2006). Contrasting findings were also reported in a study of chronic CP 55,940 injections from pd 35 to pd 45 during the prepubertal/early pubertal period, adult animals tested from pd 75 onwards showed a decrease in anxiety-related behavior (Biscaia *et al.* 2003). Unfortunately, no adult controls were added in this study. Finally, a recent study reported that acute anxiogenic effects of

THC were more pronounced in 'adult' (pd 65) than in juvenile (pd 28) male animals (Schramm-Sapota *et al.* 2007). As described before, these conflictive findings might be related to the variability of the age when animals were exposed to cannabinoids and confusion about the exact definition of developmental periods in rats (e.g. treatment before puberty onset, or use of still immature animals as adult controls).

A more detailed and not age-specific description of the general involvement of the endocannabinoid system in neuropsychiatric disorders will be given by another article in this issue (Leweke and Koethe 2008).

Problematic drug use and cannabis dependence

Regional availability of drugs and social trends have been reported to influence the prevalence of substance use disorders. However, growing evidence suggests that the developmental periods of puberty and adolescence might be primary correlates of substance use and abuse, operating across cultural trends and substances (for review see Spear 2000). High levels of novelty and sensation seeking have been proposed as strong predictors of drug and alcohol use. Therefore, it is not surprising that adolescents generally show higher rates of experimental drug use than adults, and that addictive disorders identified in adulthood most commonly originate around puberty or mid-adolescence. Most smokers, for example, begin smoking before age 18, and the onset of daily smoking is uncommon after age 25. Over 40% of adult alcoholics experience alcoholism-related symptoms between ages 15 and 19. Furthermore, an early onset of substance use predicts greater addiction severity and morbidity, including the use of multiple substances (for review see Kandel, Yamaguchi & Chen 1992; Chambers *et al.* 2003). A cross-sectional survey of adolescents between 10 and 15 years found that pubertal stage was associated with higher rates of substance abuse independent of age and school grade level. Early maturers had higher levels of substance use because they entered the risk period at an earlier point than late maturers (Patton *et al.* 2004). Similar patterns have been reported for cannabis consumption. A recent cohort study demonstrated that weekly or more frequent cannabis use in adolescents predicted a high risk for daily cannabis use in young adults, indicating that regular adolescent cannabis users appear to be on a problematic trajectory (Patton *et al.* 2007). Consistent with these findings, it has been shown that early users are in general less likely to quit their habit than those beginning at later ages (DeWit, Offord & Wong 1997).

Besides problematic drug use, the development of dependence is, in general, a completely underestimated risk of cannabis use (for further review, see also Cooper

and Haney in this issue). For a long time cannabis was not regarded as a drug of dependence, mainly because of the lack of evidence for physiological dependence (withdrawal). This view began to change in the early 1990s with the discovery of the endogenous cannabinoid system and publication of clinical and epidemiological data showing that cannabis dependence was relatively common and had important consequences (Budney & Hughes 2006). In the meantime, a specific cannabis withdrawal syndrome has been characterized (for review see Budney *et al.* 2004; Parolaro, Viganò & Rubino 2005; Budney & Hughes 2006), and it has been shown that animals and humans develop tolerance to the effects of cannabinoids (e.g. Bass & Martin 2000; Coffey *et al.* 2002; Gonzalez, Cebeira & Fernandez-Ruiz 2005). In addition, it has been demonstrated in rats that withdrawal from chronic cannabinoid exposure results in an elevation of corticotropin-releasing hormone in a similar manner as has been reported for other drugs of abuse such as opioids, psychostimulants or ethanol (Rodriguez de Fonseca *et al.* 1997; Caberlotto *et al.* 2004; Heilig & Koob 2007). Again, those who initiate cannabis consumption early during adolescence seem to have the highest risk for cannabis dependence, because an early age of onset is found to be associated with problematic cannabis use at a later age and a higher risk for dependence than an onset of cannabis consumption at older ages (Coffey *et al.* 2003; Fergusson *et al.* 2003; Chen, O'Brien & Anthony 2005). In addition, it has been shown in rats that chronic THC treatment during early puberty (pd 34–52) is not perceived as aversive, whereas an identical treatment in adult animals induces a place aversion (Quinn *et al.* 2008), suggesting that the developing brain might be less sensitive to the use-limiting aversive effects of cannabinoids and hence promote a higher vulnerability to continuous cannabis use and dependence.

Cannabis as a gateway drug

A very delicate and controversial issue is the discussion whether cannabis might act as a gateway drug, and subsequently lead to increased intake of other illicit drugs (e.g. Fergusson & Horwood 2000; Fergusson, Boden & Horwood 2006; Lynskey, Vink & Boomsma 2006). The gateway hypothesis, originally introduced by Kandel (1975), proposes the thesis that developmental stages can be observed in drug use. The sequence normally begins with the use of legal drugs, such as beer and wine, subsequently followed by hard liquor and cigarettes and proceeds in some adolescents progressively through the use of cannabis products (marijuana or hashish) to other illicit drugs such as psychostimulants and heroin.

The most striking evidence for the gateway theory of cannabis use is the observation of a temporal sequence in

the use of cannabis and other illicit drugs. There is consistent evidence that the use of cannabis almost invariably precedes the use of other illicit drugs. Additionally, there seems to be a strong association between early cannabis use and other illicit drug use, because cannabis users are more likely to use psychostimulants, hallucinogens or opioids than those who have never used cannabis (Fergusson *et al.* 2006). A USA population survey also demonstrated that those who started using cannabis at the age of 14 or younger had a higher percentage of present illicit drug abuse or dependence than those starting at older ages (SAMHSA 2004), indicating that the age of first cannabis use plays an important role in association with further drug abuse. Therefore, cannabis use might act as a causal factor and promote further illicit drug use in young people by inducing neurobiological alterations in reward-related systems in the developing brain. Conversely, it has been argued that the observed associations may simply reflect a combination of a common underlying (genetic) predisposition to substance use in general and levels of availability and access to different drug classes by contact to peer-groups or a social environment encouraging drug use and abuse and black market experiences (Fergusson & Horwood 2000; Fergusson *et al.* 2006; Hall 2006b; Lynskey *et al.* 2006).

Therefore, whether or not there is a causal relationship between cannabis use and a progression to other illicit drug use is still heavily debated (Fergusson *et al.* 2006; for detailed discussion see: Hall 2006a; Kandel, Yamaguchi & Klein 2006a; MacCoun 2006), and clarification of this contentious issue definitely requires further research. One strategy to directly evaluate the relationship of prior cannabis experience on further response to illicit drugs is the use of experimental animal models. Evidence from animal studies indicates that cannabinoids might induce lasting neuronal modulations that could alter the perception and/or reinforcing values of other drugs of abuse, independent of genetic, social or cultural factors.

Pistis *et al.* (2004) demonstrated that subchronic cannabinoid treatment (two injections of increasing doses on three consecutive days) with the synthetic cannabinoid agonist WIN induces long-lasting tolerance to acute cannabinoids in the ventral tegmental area dopaminergic neurons. When the cannabinoid treatment took place between pd 35 and pd 42, tolerance was not restricted to cannabinoids, but cross-tolerance developed to morphine, amphetamine and cocaine. This cross-tolerance was absent in adult cannabinoid-treated rats, indicating an enhanced responsiveness of the endocannabinoid system during early puberty. The mechanisms underlying the observed cross-tolerance are not known in detail yet. Notably, CB1, μ and dopamine D2 receptors share similar inhibitory G-protein systems and effectors, and

subchronic CB1 stimulation might therefore dysregulate common signaling cascades (Pistis *et al.* 2004). Furthermore, a recent study demonstrated that chronic THC administration during early puberty of male rats (pd 28–49) enhanced heroin self-administration in adulthood and increased pre-proenkephalin mRNA exclusively in the nucleus accumbens shell (Ellgren, Spano & Hurd 2007). This study therefore provides further evidence for a direct link between pubertal cannabis experience and further drug intake by demonstrating that pubertal cannabis exposure specifically and persistently alters hedonic processing, which subsequently results in enhanced opioid intake. However, a previous study by the same group failed to show an association between THC pretreatment in early adolescence (pd 28–32) and a later response to amphetamine, because no cross-tolerance or sensitization effects were found (Ellgren, Hurd & Franck 2004). A possible explanation for this apparent discrepancy might lie within the age at which cannabinoid treatment took place. THC exposure in the amphetamine study was done quite early during the juvenile phase in male rats, whereas in the heroin study and the study by Pistis *et al.* (2004), cannabinoid administration took place during early puberty and might therefore confirm the assumption that a narrow time window around puberty, as described before, represents the most vulnerable developmental period.

Taken together, these studies indicate that cannabis exposure during young ages might have a priming effect on the brain and render cannabis users more susceptible to the effects of other illicit drugs. Although strong evidence suggests that cannabinoids induce neurobiological alterations in common reward pathways during this critical period, these findings do not exclude the possibility that other factors such as genetic predisposition, social structure and environment might influence these neurodevelopmental cannabinoid effects and either enhance or attenuate further progression into illicit drug use.

CONCLUDING REMARKS

The debate about cannabis policy is often confined to two conflicting opinions: either it is claimed to legalize cannabis because its use is harmless and useful for therapeutic purposes, or strict prohibition of its use is requested because cannabis is harmful (Hall 2006b). This rather emotional quarrel between trivialization and demonization of cannabis products often prevents an objective discussion of the possible specific risks of cannabis exposure for young consumers. Young cannabis users might be more vulnerable to residual cognitive impairments, have a higher risk for neuropsychiatric disorders, in particular when genetic or environmental predispositions exist, and might be more susceptible to further illicit drug use and

cannabis dependence. The neurobiological mechanisms mediating the specific vulnerability of teenage brains to cannabinoid exposure are not yet known in detail and the association between early cannabis use and subsequent problems may be due partially to common risk factors. Nevertheless, the human studies as well as the data from animal research reviewed in the present article all point out clearly that the age in which an individual is exposed to cannabinoids has a high impact on the subsequent effects of this drug. Taken together, these findings suggest that young people, in particular during the susceptible period around pubertal development, represent a highly vulnerable cannabis consumer group and seem to be at a higher risk of suffering from adverse consequences of cannabinoid exposure than adult consumers. Hence, there is an urgent need for long-term follow-up studies and further animal research for clarifying the exact neurobiological mechanisms, detailed consequences and possible additional risk factors for deleterious cannabinoid effects during puberty.

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