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Research Report

Behavioural disturbances and altered Fos protein expression in adult rats after chronic pubertal cannabinoid treatment

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ABSTRACT

Cannabis is one of the world's most popular recreational drugs. However, little is known about long-lasting cellular and neurobehavioural effects of chronic cannabinoid intake, especially during puberty where cannabis use among humans is commonly initiated. This study in rats investigates the long-term effect of pubertal cannabinoid treatment on prepulse inhibition (PPI), locomotor activity and on anxiety in the elevated-plus maze during adulthood. Furthermore, changes in adult basic neuronal activity, assessed by c-Fos immunoreactivity (Fos IR), and a potentially altered Fos expression after acute treatment with dopaminergic drugs was evaluated. Chronic treatment with the synthetic cannabinoid full agonist WIN 55,212-2 (WIN; 1.2 mg/kg) was carried out over 25 days of the rats' puberty and subsequent behavioural testing was conducted in adult animals. Finally, Fos IR was evaluated in several brain regions under basal conditions and after acute administration of haloperidol (0.1 mg/kg) and apomorphine (2 mg/kg). Chronic WIN treated animals exhibited a lasting disruption of PPI. These rats were also more active in the open field and less anxious in the elevated-plus maze than their vehicle treated controls. Additionally, when comparing Fos IR in selected brain regions, these animals displayed altered basal neuronal activity and responded differently to acute application of haloperidol or apomorphine. Taken together, these results indicate that chronic stimulation of the cannabinoid receptor CB_1 during the rats' puberty not only leads to persistent behavioural changes but also to cellular long-term adaptations within brain regions critical for drug of abuse or neuropsychiatric diseases.

1. Introduction

Products of the hemp plant *Cannabis sativa*, marijuana and hashish, belong to the most widely used illicit substances worldwide and cannabis potency as well as the rate of abuse are increasing since the early 1990s (UNODC, 2007). Despite its long history as a drug for both recreational and medicinal purposes, very little is known about potentially persistent effects of chronic cannabis use, such as long-term neurobehavioural disturbances and/or neuronal adaptations. Nowadays, most cannabis users start in their mid to late teens which coincides with major neuronal changes in the central nervous system (Monshouwer et al., 2005; Hall, 2006; EMCDDA, 2007). Hence concern has been growing about the possible adverse effects of cannabis on physical and/or mental health.

It has been shown in humans that cannabinoid exposure especially during pubertal development induces lasting behavioural and morphological alterations (Pope et al., 2003;

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Arseneault et al., 2004; Caspi et al., 2005). For example, Ehrenreich et al. (1999) found that early-onset cannabis users (before the age of 17 years) displayed a disruption of attentional functions. Additionally, another study revealed that young consumers had a higher percentage of white matter relatively to whole-brain volume (Wilson et al., 2000). In both studies no such persistent effects were found in control subjects, i.e. long-term cannabis users who had initiated use after the age of 17.

These findings are supported by animal studies, which offer better possibilities for a controlled investigation of the neuroactive components of cannabis' impact on brain maturation. Treatment of rats with the synthetic cannabinoid receptor agonist WIN 55,212-2 (WIN) or CP 55,940 throughout pubertal development from postnatal day (pd) 40 to pd65 (Schneider, 2008) led to lasting behavioural alterations in adulthood such as attentional deficits, impaired memory and altered social behaviour. Prepubertal (pd 15–40) or adult (>pd70) cannabinoid treatment had no such effects or affected the rats' behaviour only marginally (Schneider and Koch, 2003, 2005; O'Shea et al., 2004, 2006).

Interestingly, in a CB_1 receptor radioligand binding study Rodriguez de Fonseca et al. (1993) found that the endocannabinoid system, which consists of endogenous cannabinoids (e.g. anandamide) and at least two cannabinoid receptors, the neuronal CB_1 receptor and the CB_2 receptor (which is mainly expressed in the immune system), displays its developmental peak at the onset of puberty. Additionally, during this period numerous neurodevelopmental alteration take place, e.g. myelination, synaptic pruning and the maturation of neurotransmitter systems such as the dopaminergic and glutamatergic system (Andersen et al., 2000; Spear, 2000; Schneider, 2008).

It has been a matter of debate whether especially adolescent cannabis consumption increases the risk for certain neuropsychiatric diseases such as schizophrenia (Arseneault et al., 2004; Caspi et al., 2005). Increased levels of endocannabinoids were found in the cerebrospinal fluid of schizophrenic patients (Leweke et al., 1999, 2007) as well as alterations in prefrontal CB_1 receptor density (Dean et al., 2001), indicating a connection between early cannabis use and schizophrenia. Additionally, there seems to be an association between time and frequency of cannabis use and subsequent use of other illicit drugs. This cannabis "gateway hypothesis" is currently subject to controversial discussions, but human studies indicate that young cannabis users are more likely to use psychostimulants, hallucinogens or opioids than those who have never used cannabis (Ferrguson et al., 2006). Further evidence comes from experimental animal models which showed that cannabinoids might induce lasting neuronal alterations that could affect the stimulant and/or reinforcing values of other drugs of abuse (Pistis et al., 2004; Ellgren et al., 2007). Therefore, both human and animal studies indicate puberty as a developmental period during which the immature organism is vulnerable to the long-term adverse effects of exogenous cannabinoids.

The aim of the present study in rats was to identify long-term effects of pubertal WIN treatment on sensorimotor gating, motor activity, and anxiety during adulthood. Additionally, altered adult neuronal activity in response to chronic WIN as well as dopaminergic drugs was assessed via c-Fos immunoreactivity (Fos IR).

The acoustic startle response (ASR) can be elicited in both humans and rodents by a sudden, intense noise pulse and is characterised by a fast contraction of facial and skeletal muscles as well as by an arrest of ongoing behaviours (Koch, 1999). If the startling stimulus is shortly preceded by a weaker, non-startling stimulus (prepulse), the magnitude of the ASR is reduced. This phenomenon is called prepulse inhibition (PPI) and is thought to be due to a pre-attentive filter or sensorimotor gating mechanism regulated by a modulatory circuitry involving cortico-limbic brain structures (Swerdlow et al., 2001; Koch and Fendt, 2003). Interestingly, a reduced PPI can be observed in certain neuropsychiatric disorders, e.g. schizophrenia (Swerdlow et al., 2006). In humans and rodents PPI deficits can be induced by various drugs (reviewed in Geyer et al., 2001) as well as CB_1 receptor agonists (Schneider and Koch, 2002; Wegener et al., 2008). However, studies investigating the long-term effects of pubertal cannabinoid treatment on sensorimotor gating ability are rare and provide inconsistent results. One previous study from our laboratory has shown that chronic pubertal but not adult WIN treatment led to persistent PPI deficits in Wistar rats (Schneider and Koch, 2003). In contrast, one other study found no such differences when using Sprague–Dawley rats and a slightly different experimental setup (Bortolato et al., 2005). Therefore, we strived to replicate our previous data as well as to add more information about the behavioural consequences of chronic cannabinoid exposure during a critical developmental stage.

Besides PPI regulating circuits, the CB_1 receptor is densely expressed in regions which initiate and coordinate movement, e.g. the cerebellum and the basal ganglia, and can also be found in limbic structures, e.g. amygdala, which is known to mediate anxiety-like behaviour (Herkenham et al., 1991; Tsou et al., 1998). To assess the impact of pubertal WIN on adult locomotor activity and anxiety we also tested the rats' behaviour in the open field and in the elevated-plus maze.

Finally, altered neuronal activity as consequence of chronic pubertal cannabinoid treatment was evaluated in adult rats. It was suggested that expression of the proto-oncogene c-*fos* may serve as a marker for neuronal activity (Dragunow and Faull, 1989; Sagar et al., 1988). The Fos protein is a well characterised member of a family of immediate early genes (IEG) which can bind to DNA, thereby influencing the transcription of specific target genes (Sheng and Greenberg, 1990). Altered expression of such IEGs would lead to altered neuronal activity and ultimately to changes in the neural circuits in which those neurons operate. Psychostimulant drugs such as cocaine or amphetamine, but also antipsychotic drugs are known to induce a specific pattern of Fos activation in the striatum and nucleus accumbens (NAc) (reviewed in Harlan and Garcia, 1998; Sumner et al., 2004). Previous studies examining the effects of acute Δ^9-THC or its synthetic analogues on Fos IR have also shown a consistent distribution of Fos expression, e.g. in the caudate–putamen, NAc, ventral tegmental area (VTA), and amygdala (McGregor et al., 1998; Miyamoto et al., 1997; Arnold et al., 2001; Patel and Hillard, 2003; Rubino et al., 2007).

However, little is known about possible persistent changes in neuronal activity after chronic pubertal CB_1 receptor