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Chronic cannabinoid exposure during adolescence leads to long-term structural and functional changes in the prefrontal cortex

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Abstract
In many species, adolescence is a critical phase in which the endocannabinoid system can regulate the maturation of important neuronal networks that underlie cognitive function. Therefore, adolescents may be more susceptible to the neural consequences of chronic cannabis abuse. We reported previously that chronically exposing adolescent rats to the synthetic cannabinoid agonist CP55,940 leads to impaired performances in adulthood *i.e.* long-lasting deficits in both visual and spatial short-term working memories. Here, we examined the synaptic structure and function in the prefrontal cortex (PFC) of adult rats that were chronically treated with CP55,940 during adolescence. We found that chronic cannabinoid exposure during adolescence induces long-lasting changes, including (1) significantly altered dendritic arborization of pyramidal neurons in layer II/III in the medial PFC (2) impaired hippocampal input-induced synaptic plasticity in the PFC and (3) significant changes in the expression of PSD95 (but not synaptophysin or VGLUT3) in the medial PFC. These changes in synaptic structure and function in the PFC provide key insight into the structural, functional and molecular underpinnings of long-term cognitive deficits induced by adolescent cannabinoid exposure. They suggest that cannabinoids may impede the structural maturation of neuronal circuits in the PFC, thus leading to impaired cognitive function in adulthood.

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1. Introduction

Chronic cannabis abuse during adolescence can impair cognitive functioning (Meier et al., 2012) and significantly increases the long-term risk of psychiatric disease, including schizophrenia (Arseneault et al., 2004). Imaging studies revealed that repeated cannabis use during adolescence induces gray-matter changes in the prefrontal and temporal cortices (Batalla et al., 2013) and causes abnormal connectivity, particularly in hippocampal afferent fibers (Zalesky et al., 2012). However, the precise cellular changes that underlie these effects are poorly understood. Animal studies have shown that adolescent exposure to cannabinoids induces persistent behavioral abnormalities that resemble psychotic-like symptoms, including anhedonia and impaired cognitive processes, sensorimotor gating, and social interactions (Renard et al., 2014; Schneider and Koch, 2003). In addition, cortical oscillations can be irreversibly suppressed in brain areas that developed poorly during adolescence, including the prefrontal cortex (PFC) (Raver and Keller, 2014).

Adolescence is a critical developmental phase during which the cortical and limbic circuits are extensively remodeled, leading to the acquisition of mature behavior and the emergence of adult cognitive processes (Andersen, 2003). This remodeling includes changes in dendritic growth, dendritic spine density, myelination, synaptic pruning, receptor distribution, volumetric growth and the programming of neurotrophic levels (Spear, 2000).

During adolescence, the endocannabinoid system is highly active, and cannabinoid type 1 receptors (CB1Rs) undergo extensive reorganization (Ellgren et al., 2008; Heng et al., 2011), particularly in the PFC and limbic circuit (Heng et al., 2011). To drive central nervous system maturation, the endocannabinoid system plays a pivotal role by acting on the various neurotransmitter systems that regulate neurogenesis, differentiation and synaptogenesis (Harkany et al., 2008). Thus, adolescent exposure to exogenous cannabinoids might interfere with normal cortical maturation, causing neurobiological changes that impair brain function. Indeed, we previously reported that chronic adolescent exposure of the synthetic CB1R agonist CP55,940 (CP) induces long-lasting deficits in short-term memory and spatial working memory in rats (Renard et al., 2013). Given the well-established role of both the hippocampus and the PFC in learning and memory (Churchwell and Kesner, 2011; Floresco et al., 1997), it is likely that the emergence of cognitive deficits following chronic adolescent cannabinoid exposure is due to a perturbation in the function and structure of prefrontal and hippocampal networks. However, in general insufficient direct evidence supports this hypothesis and in particular, specific and lasting cannabinoid-induced structural and functional changes in the PFC remain poorly identified.

Here, as a first step to characterize the impact of adolescent cannabinoid exposure on adult PFC structure and function, we assessed the dendritic morphology in the PFC and measured synaptic plasticity in the hippocampus-PFC network in adult rats, which were chronically exposed to a synthetic cannabinoid agonist during adolescence. Because the rates of cannabis use in humans increase during the transition from adolescence to young adulthood, we have chosen to expose the animals to CP treatment on postnatal days (P) 29–50, a period described as early and middle adolescence (Laviola et al., 2003). The age span chosen extends beyond the prototypic adolescent period (P28–42), which starts around 10 days before puberty and ends a few days after (Spear, 2000), to reach late adolescence on the border to young adulthood (Andersen, 2003). We found that chronic adolescent cannabinoid exposure alters both dendritic morphology of pyramidal neurons and the expression of the postsynaptic marker PSD95 protein in the PFC and induces hippocampus-PFC network plasticity changes in adult rats.

2. Experimental procedures

2.1. Animals

Male Wistar Han rats (77–128 g) were obtained from Janvier Labs (Le Genest St Isle, France) at 22 days of age (postnatal day 22, or P22). The rats were group-housed under standard conditions in Macrolon cages at four rats per cage. The rats were housed on a 12 h/12 h light/dark schedule (with the lights on from 7:00 to 19:00) and were given free access to food and water. All procedures were performed in accordance with National (JO 887-848) and European (86/609/EEC) legislation regarding animal experimentation.

2.2. Drug preparation and administration

CP55,940 ((-)cis-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-Trans-4-(3-hydroxypropyl) cyclohexanol) (CP; Tocris Cookson, Bristol, UK) was dissolved in DMSO (to 10% of the final volume), suspended in Tween-80 (to 20% of the final volume), and diluted in physiological saline (0.9% NaCl). All injections were administered intraperitoneally at a volume of 1 ml/kg body weight. The adolescent exposure experiment began at P29. During the 3-week treatment period from P29 through P50, the animals received daily injections of CP at increasing doses (0.15 mg/kg for seven days, 0.20 mg/kg for seven days, then 0.30 mg/kg for seven days); the control group received injections of the vehicle (VEH). The experimental protocol is illustrated in Figure 1. Based on previous studies, these specific CP doses are known to produce long-term behavioral impairments in rats (O'Shea et al., 2006). The doses were increased gradually in order to minimize the development of drug tolerance (González et al., 2005).

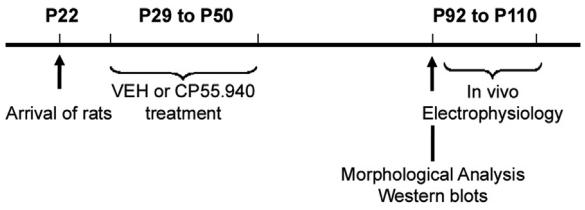

Figure 1 Schematic overview of the experimental protocol, including cannabinoid (CP55,940 or vehicle; VEH) exposure during adolescence and morphological, western blots analysis and *in vivo* electrophysiology performed in adult rats. P = postnatal day.

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