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Research Report

Delta-9-tetrahydrocannabinol disrupts hippocampal neuroplasticity and neurogenesis in trained, but not untrained adolescent Sprague-Dawley rats

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ABSTRACT

Cannabis is the most widely used illicit drug, and disruption of learning and memory are commonly reported consequences of cannabis use. We have previously demonstrated a spatial learning impairment by Δ^9-tetrahydrocannabinol (THC) in adolescent Sprague-Dawley rats (Steel et al., 2011). The molecular mechanisms underlying behavioural impairment by cannabis remain poorly understood, although the importance of adaptive changes in neuroplasticity (synaptic number and strength) and neurogenesis during learning are accepted. Here we aimed to identify any effects of THC on the early induction of these adaptive processes supporting learning, so we conducted our analyses at the mid-training point of our previous study. Both untrained and trained (15 days of training) adolescent (P28–P42) Sprague-Dawley rats were treated daily with THC (6 mg/kg i.p.) or its vehicle, and changes in the levels of markers of hippocampal neuroplasticity (CB1R, PSD95, synapsin-I, synapsin-III) and neurogenesis (Ki67, DCX, PSA-NCAM, BrdU labelling) by training were measured. Training of control animals, but not THC-treated animals increased neuroplasticity marker levels. However training of THC-treated animals, but not control animals reduced immature neuronal marker levels. Levels of hippocampal proliferation, and survival of the BrdU-labelled progeny of these divisions were unaffected by THC in trained and untrained animals. These data show a smaller neuroplastic response, and a reduction of new-born neuronal levels not attributable to effects on proliferation or survival by THC-treatment during training. Importantly no effects of THC were seen in the absence of training, indicating that these effects represent specific impairments by THC on training-induced responses.

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1. Introduction

Cannabis use peaks during adolescence, with emerging trends for a younger age of initiation and a greater disregard for its potential harmful effects amongst youths. This is despite earlier adolescent onset of cannabis use being associated with greater decline in cognitive functions, including learning and memory performance in both human and rodent models. The cannabinoid receptor (CB1R) is widely expressed in the brain and is involved in several developmental systems, including modulation of neuronal connectivity and neurogenesis. The on-going nature of cognitive impairments associated with adolescent cannabis exposure makes the understanding of how cannabis causes its behavioural effects important (Fergusson and Boden, 2008; Harvey et al., 2007; Meier et al., 2012; Soderstrom and Gilbert, 2013; Trezza et al., 2008; Trezza et al., 2012).

The hippocampus is the most relevant brain structure for spatial learning (Moser et al., 1993; Pearce et al., 1998; Richmond et al., 1999; Talpos et al., 2008) and it expresses high levels of CB1R that peak during adolescence (Soderstrom and Gilbert, 2013). Administration of Δ^9-tetrahydrocannabinol (THC) reduces neurotransmitter release, alters the activity of several receptor systems and perturbs second messenger signalling in the hippocampus (Fishbein et al., 2012; Long et al., 2013; Mateos et al., 2011; Trezza et al., 2012). Perhaps unsurprisingly, cannabinoids disrupt long-term potentiation (LTP) in hippocampal neurons (Collins et al., 1995; Misner and Sullivan, 1999; Terranova et al., 1995).

While changes in the underlying physiology of the hippocampus may certainly contribute to the cognitive deficits from cannabis use, few studies have investigated the effects of THC on training-induced hippocampal molecular plasticity that supports learning. The recruitment of new synapses, as well as increased transcription and translation of synaptic signalling components are important events supporting the increased LTP that is critical to learning (Bailey and Kandel, 1993; Hicks et al., 1997; Moser, 1999; Muller et al., 2000). Rubino et al. (2009) found that learning impairment in adults following THC-treatment during adolescence was associated with a deficit in establishment and/or function of synapses (neuroplasticity) using a rodent model. It was not clear in their study whether THC specifically impaired the learning-enhanced establishment of synapses and plasticity, or rather caused a general deficiency in neuroplasticity markers that has a secondary effect on learning.

Hippocampal neurogenesis makes a crucial contribution to spatial learning. Hippocampal new-born neurons are preferentially incorporated into memory circuits where they enhance memory encoding (Deng et al., 2010). New-born neurons are thus required for, and their survival enhanced by, learning (Gould et al., 1999; Shors et al., 2001; Sun et al., 2004). Incorporation of too many immature neurons may lead to excessive hippocampal activity and memory interference, thus enhanced neuronal survival by training may be balanced by reduced proliferation of progenitors to maintain an optimum activity level (Deng et al., 2010; Epp and Galea, 2009). Treatment with THC appears to have little effect on hippocampal proliferation and neuronal survival in untrained animals (Kochman et al., 2006; Wolf et al., 2010), although few studies have investigated the effect of cannabinoids on any aspect of changes in neurogenesis during learning.

In a previous study we showed that THC causes a spatial learning impairment in adolescent Sprague-Dawley rats after 27 days of training (Steel et al., 2011). In the present study, using our published spatial training protocol, we investigate early effects of THC-treatment on changes in neuroplasticity and neurogenesis during training. Animals treated with THC do eventually learn this task, suggesting a permissive molecular response is lacking initially but eventually achieved. Our aim was to identify these early effects of THC on the induction of molecular processes supporting learning, so we conducted our analyses mid-training before the emergence of a measurable learning impairment. We included untrained animals in our analysis to differentiate between general deficiencies caused by adolescent THC exposure that affect learning, and any specific impairments of training-mediated mediated responses by THC. We investigated neuroplasticity using presynaptic (CB1R, synapsin-I, synapsin-III) and post-synaptic markers (postsynaptic density protein 95, PSD95) that are important in synapse establishment and function (Feng et al., 2002; Heifets and Castillo, 2009; Kennedy, 2000; Martin et al., 2000; Sudhof et al., 1989). Neurogenesis was assessed at the level of proliferation (Ki67), survival (BrdU labelling) and the level of immature, new-born neuronal markers doublecortin (DCX) and the polysialiated neural cell adhesion molecule (PSA-NCAM) (Scholzen and Gerdes, 2000; von Bohlen und Halbach, 2007).

2. Results

We previously described a method by which spatial learning in adolescent Sprague-Dawley rats is impaired by THC after 27 days training (Steel et al., 2011). The learning impairment in our previous study was not measureable after only 15 days training, nor was any behavioural impairment by THC yet present after 15 days training as in this study (data not shown). We performed our analyses at this point because THC-treated animals do eventually learn the task, suggesting a permissive molecular response is eventually achieved but is initially lacking. We aimed to determine whether THC-treatment was associated with an impairment of plasticity and neurogenesis in these initial stages of training.

2.1. *Neuroplasticity*

Using CB1R, PSD95, synapsin-I and synapsin-III as markers, we investigated how THC may affect the molecular neuroplasticity supporting learning. We sought to investigate whether THC impaired the adaptive neuroplasticity in response to training, and whether any impairment by THC was also seen in the absence of training (Fig. 1).

Training of vehicle-treated animals increased the expression of mRNA for all neuroplasticity markers, especially CB1R ($p<0.01$), synapsin-I ($p<0.01$) and synapsin-III ($p<0.001$). An increase in PSD95 by training was also evident, but greater variability in the data precluded statistical significance for this marker. In THC-treated animals there was no significant