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Cortical neuroinflammation contributes to long-term cognitive dysfunctions following adolescent delta-9-tetrahydrocannabinol treatment in female rats

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Abstract
Over 180 million people consume cannabis globally. Cannabis use peaks during adolescence with a trend for continued consumption by adults. Notably, several studies have shown that long-term and heavy cannabis use during adolescence can impair brain maturation and predispose to neurodevelopmental disorders, although the neurobiological mechanisms underlying this association remain largely unknown.

In this study, we evaluated whether, in female rats, chronic administration of increasing doses of the psychotropic plant-derived cannabis constituent, delta-9-tetrahydrocannabinol (THC), during adolescence (PND 35–45) could affect microglia function in the long-term. Furthermore, we explored a possible contribution of microglia to the development of THC-induced alterations in mood and cognition in adult female rats.

Present data indicate that adolescent THC administration induces a persistent neuroinflammatory state specifically localized within the adult prefrontal cortex (PFC), characterized by increased expression of the pro-inflammatory markers, TNF-α, iNOS and COX-2, and reduction of the anti-inflammatory cytokine, IL-10. This neuroinflammatory phenotype is associated with down-regulation of CB1 receptor on neuronal cells and up-regulation of CB2 on microglia cells, conversely. Interestingly, blocking microglia activation with ibudilast during THC treatment significantly attenuates short-term memory impairments in adulthood, simultaneously preventing the increases in TNF-α, iNOS, COX-2 levels as well as the up-regulation of CB2 receptors on microglia cells. In contrast, THC-induced depressive-like behaviors were unaffected by ibudilast treatment.

Our findings demonstrate that adolescent THC administration is associated with persistent

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neuroinflammation within the PFC and provide evidence for a causal association between microglial activation and the development long-term cognitive deficits induced by adolescent THC treatment.

1. Introduction

While transient inflammatory responses in the central nervous system (CNS) are generally beneficial to the host, chronic neuroinflammation is maladaptive and can profoundly affect neuronal plasticity and brain homeostasis. Indeed, neuroinflammation is present in several chronic psychiatric conditions and can directly influence neurotransmitter activity and synaptic plasticity, as well as regulation of cognition and mood (Najjar et al., 2013).

As the primary resident immune cells in the brain, microglia are obvious candidate mediators of abnormal brain-immune dialogue in psychopathology. Microglia in the healthy brain interact with neuronal and non-neuronal elements, both structurally and functionally, to influence neuronal plasticity and function directly or indirectly (Paolicelli and Gross, 2011; Schafer et al., 2013; Tremblay, 2011), suggesting that microglial dysregulation could play a role in the pathogenesis of neuropsychiatric and neurologic disorders, as supported by human and animal studies (Frick et al., 2013).

Adolescence is an important developmental phase characterized by strong neurobehavioral plasticity and many maturation processes in the CNS. Prominent neuronal modifications occur in brain regions such as the prefrontal cortex (PFC) and limbic areas (Spear, 2000) and are believed to support the emergence of adult behavior and cognitive functions (Spear, 2000; Andersen, 2003). The contribution of microglia in the shaping of neuronal circuits appears to be particularly important in this critical time-window, during which adverse events can have significant and enduring effects on the development of the CNS, and, as a consequence, on behavior later in life (Schafer et al., 2013).

The incidence of substance use and abuse is more prevalent during adolescence than any other age (Chambers et al., 2003). Cannabis is the most commonly used illicit drug among adolescents (Johnston et al., 2012) and evidence from human studies and animal models suggests that early onset of Cannabis use can increase risks for cognitive dysfunction, CNS changes and neuropsychiatric disorders in adulthood (Renard et al., 2014).

In line with this, work from our lab demonstrates that chronic THC exposure during adolescence has long-term sex-dependent influence on measures of depressive- and psychotic-like behaviors in female rats (Realini et al., 2011; Rubino et al., 2008; 2015; Rubino and Parolaro, 2015; Zamberletti et al., 2014). In contrast, no behavioral changes develop when the same treatment protocol is performed in adulthood (Realini et al., 2011), indicating that the effect of THC are both sex- and age-dependent. In females, the behavioral phenotype is associated with profound alterations in the endocannabinoid, glutamate and GABA neurotransmitter systems within the PFC (Rubino et al., 2015; Zamberletti et al., 2014), suggesting that exposure of adolescent female rats to THC at certain times and at certain doses can lead to PFC circuitry abnormalities.

Given that microglia are in the position of being particularly vulnerable to developmental disturbances and microglia activation is often associated with neuropsychiatric conditions, we hypothesized that (1) chronic THC exposure during adolescence could have long-term consequences on microglia functions and (2) microglia activation could contribute to the development of THC-induced alterations in mood and cognition in adult female rats.

In the present study, we provide the following evidence in support of these hypotheses: (1) adolescent THC treatment is associated with long-term microglia activation specifically within the PFC, as demonstrated by increased expression of the microglia marker, Iba1, altered microglia morphology, dysregulations in several pro- and anti-inflammatory mediators as well as up-regulation of microglial CB2 receptors. (2) Chronic administration of Ibudilast, a compound known to attenuate microglia proinflammatory responses, concomitantly to THC during adolescence reduced both the neuroinflammatory picture in the PFC and the cognitive deficit induced by adolescent THC treatment.

2. Experimental procedures

2.1. Animals

Female Sprague Dawley rats aged 28 days at the time of arrival were obtained from Charles River laboratories (Calco, Italy) and were housed in clear plastic cages on a 12 h light-dark cycle (lights on 08:00h) and in a temperature (22 ± 2 °C) and humidity controlled environment ($50 \pm 10\%$) with a plastic tube for environmental enrichment. All animals had free access to food and water. All experiments took place during the light phase and were performed in accordance with the guidelines released by the Italian Ministry of Health (D.L. 2014/26), and the European Community directives regulating animal research (2010/63/EU). All efforts were made to minimize the number of animals used and their suffering.

2.2. Adolescent THC treatment

Delta-9-tetrahydrocannabinol (THC), a generous gift from GW Pharmaceutical (Salisbury, UK), was further purified to reach THC concentrations as high as 90% and dissolved in ethanol, cremophor and saline (1:1:18). The experiments were carried out according to the timeline reported in Figure 1. Rats were injected with increasing doses of THC, or vehicle, twice a day from PND 35 to PND 45 (2.5 mg/kg, PND 35–37; 5 mg/kg, PND 38–41; 10 mg/kg, PND 42–45), according to our previous published protocol (Realini et al., 2011; Rubino et al., 2008; Zamberletti et al., 2014).