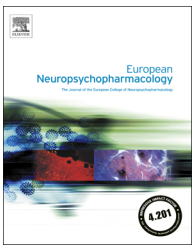
European Neuropsychopharmacology (2014) 24, 974–985



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Juvenile cannabinoid treatment induces frontostriatal gliogenesis in Lewis rats

Marco Bortolato[a,e,*], Valentina Bini[b], Roberto Frau[b], Paola Devoto[b], Alessandra Pardu[b], Yijun Fan[c], Marylou V. Solbrig[c,d,**]

[a]*Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, 1251 Wescoe Hall Dr, Malott Hall, Room 5040, Lawrence, KS, USA*
[b]*"Guy Everett" Laboratory, Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy*
[c]*Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada*
[d]*Department of Medicine (Neurology), University of Manitoba, Winnipeg, MB, Canada*
[e]*Consortium for Translational Research on Aggression and Drug Abuse (ConTRADA), University of Kansas, Lawrence (KS), USA*

Received 23 May 2013; received in revised form 20 November 2013; accepted 11 December 2013

KEYWORDS
Cannabinoids;
Adolescent exposure;
Dopamine;
Gliogenesis;
Lewis rats

Abstract
Cannabis abuse in adolescence is associated with a broad array of phenotypical consequences, including a higher risk for schizophrenia and other mental disturbances related to dopamine (DA) imbalances. The great variability of these sequelae likely depends on the key influence of diverse genetic vulnerability factors. Inbred rodent strains afford a highly informative tool to study the contribution of genetic determinants to the long-term effects of juvenile cannabinoid exposure. In this study, we analyzed the phenotypical impact of the synthetic cannabinoid agonist WIN 55,212-2 (WIN; 2 mg/kg/day from postnatal day 35–48) in adolescent Lewis rats, an inbred strain exhibiting resistance to psychotomimetic effects of environmental manipulations. At the end of this treatment, WIN-injected animals displayed increased survival of new cells (mainly oligodendroglia precursors) in the striatum and prefrontal cortex (PFC), two key terminal fields of DAergic pathways. To test whether these changes may be associated with enduring behavioral alterations, we examined the consequences of adolescent WIN treatment in adulthood (postnatal days 60–70), with respect to DA levels and metabolism as well as multiple behavioral paradigms. Rats injected with WIN exhibited increased turnover, but not levels, of striatal DA. In addition, cannabinoid-treated animals displayed increases in acoustic startle latency and novel-object exploration; however, WIN treatment failed to induce overt deficits of sensorimotor gating and social

*Corresponding author.
**Corresponding author at: Department of Medicine (Neurology), University of Manitoba, GF543 HSC, 820 Sherbrook Street, Winnipeg, Manitoba R3A 1R9, Canada.
E-mail addresses: bortolato@ku.edu (M. Bortolato), msolbrig2@hsc.mb.ca (M.V. Solbrig).

0924-977X/$ - see front matter
http://dx.doi.org/10.1016/j.euroneuro.2013.12.011

interaction. These results indicate that, in Lewis rats, juvenile cannabinoid exposure leads to alterations in frontostriatal gliogenesis, as well as select behavioral alterations time-locked to high DAergic metabolism, but not overt schizophrenia-related deficits.

1. Introduction

Cannabis is the most widely abused illicit substance in Western countries (UNODC, 2012). Over the past decade, the recreational use of marijuana and other cannabis preparations has particularly grown in popularity among teenagers, and the age of first use has concurrently declined (Hall, 2006). This scenario is particularly concerning, since adolescent abuse of marijuana has been recently shown to increase schizophrenia risk in vulnerable individuals (Henquet et al., 2005). The link between juvenile cannabis abuse and schizophrenia has also been confirmed by animal studies. In Wistar rats, administration of the potent cannabinoid agonist WIN 55,212-2 (WIN) in adolescence has been shown to produce a number of enduring behavioral aberrations reminiscent of schizophrenia-related endophenotypes and symptoms, including sensorimotor gating deficits, as well as social and mnemonic impairments (Schneider and Koch, 2003; Schneider et al., 2008). These alterations may be underpinned by alterations in the dopamine (DA) system, a key substrate in the pathophysiology of schizophrenia (Howes and Kapur, 2009). In line with this possibility, early cannabinoid exposure has been shown to reduce the responsiveness of DAergic neurons to the stimulating action of cannabinoids (Pistis et al., 2004).

Whereas only a minority of young marijuana smokers develop schizophrenia, other individuals may exhibit different psychopathological sequelae, such as mood and personality disorders, anxiety or attention impairments (Degenhardt et al., 2012; for a general overview, see Jager and Ramsey, 2008). The heterogeneity of these clinical consequences is partially dependent on the different genetic characteristics of the smokers (Hollis et al., 2008). Thus, understanding the neurobiological substrates of these variations (and more specifically of schizophrenia resistance) is a fundamental goal for the development of effective preventive or therapeutic interventions to reduce the risk or severity of schizophrenia or other related neuropsychiatric disorders.

Previous studies have shown that Lewis rats, an inbred strain derived from Wistar stock, display high sensitivity to DA-releasing properties of cannabinoids (Chen et al., 1991) but are typically resistant to the schizophrenia-related effects of multiple environmental and pharmacological manipulations (Lipska and Weinberger, 1996; Swerdlow et al., 2004; Varty and Geyer, 1998). Based on these results, we hypothesized that cannabinoid treatment of adolescent Lewis rats would not result in schizophrenia-related phenotypes (such as social deficits and sensorimotor gating impairments), but rather in distinct phenotypical changes related to different functional and behavioral domains. Thus, in the present study we examined the impact of adolescent administration of WIN in Lewis rats. In particular, we analyzed the long-term consequences of WIN administration on behavioral functions associated with schizophrenia and DA functional roles, such as sensorimotor gating, social interaction and exploratory behavior; in addition, we measured DA levels and metabolism in the prefrontal cortex (PFC) and striatum, two major terminal areas of DAergic fibers whose functional and morphological deficits have been linked to schizophrenia (Abi-Dargham et al., 1998; Broadbelt et al., 2002; Callicott et al., 2000; Meyer-Lindenberg et al., 2002; Pantelis et al., 1997). Recent studies suggest that DA dysregulations may lead to schizophrenia through altered modulation of progenitor stem cells in the subventricular zone (SVZ) (Inta et al., 2011; O'Keeffe et al., 2009), which may in turn affect the production of glia and neurons in the PFC and striatum (Li et al., 2010; Suzuki and Goldman, 2003); accordingly, schizophrenia features alterations in glia and neurons in these two regions (Beckmann and Lauer, 1997; Rajkowska et al., 2002; Uranova et al., 2007). Thus, we also tested whether adolescent WIN treatment may also affect gliogenesis and/or neurogenesis in these regions and SVZ of Lewis rats.

2. Experimental procedures

2.1. Animals

Male Lewis rats (Charles River Labs, Wilmington, MA, USA) were housed in groups (4 per cage) and maintained in temperature- and humidity-controlled rooms, on a 12-h light–dark cycle (with lights off from 7 AM to 7 PM). Food and water were provided ad libitum and all efforts were made to minimize the number of animals used and their suffering. All procedures were approved by the Institutional Animal Care and Use Committees at the Universities of Cagliari and Manitoba, and performed according to the guidelines of the Policy for the Humane Care and Use of Laboratory Animals.

2.2. Drugs and treatments

Either non-selective cannabinoid receptor agonist R(+)-WIN 55,212-2 (WIN) (Sigma, St. Louis, MO, USA), or its vehicle (Tween 80 in 0.9% saline solution, 20:80 vol:vol) was administered for 2 weeks, from postnatal day 35 to day 48 (P35–P48). The daily dose of WIN was 2 mg/kg (intraperitoneal, i.p.), divided in two doses of 1 mg/kg injected every 12 h. This administration regimen was used to stimulate a high-dose, frequent consumption of cannabis during juvenile age. The survival of newly born cells (BrdU-labeled at the start of the experiment) was analyzed in a subset of animals sacrificed at the end of WIN treatment (P48). The assessment of brain DA levels and behavioral responses were carried out on separate batches of animals ($N=30$) between P60 and P70.

2.3. Neuro- and gliogenesis

2.3.1. Bromodeoxyuridine (BrdU) incorporation

Rats were treated with three 50 mg/kg ip injections of the S phase marker 5-bromo-2′-deoxyuridine (BrdU) (Sigma, St. Louis, MO, USA) at 6 h intervals at 34 days of age, on the day prior to the first WIN or vehicle treatment. At the end of WIN regimen, animals were