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The effects of adolescent cannabinoid exposure on seizure susceptibility and lethality in adult male rats

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ABSTRACT

There is substantial evidence in rodent models that chronic exposure to cannabinoids during adolescence can alter the development of neurobiological systems that are implicated in regulating brain activity and seizure. The current study explored whether adolescent cannabinoid treatment affects subsequent, adult seizure susceptibility. Sixty male Wistar Kyoto rats were treated with either the synthetic cannabinoid, CP 55,940 (0.4 mg/kg, one treatment per day), or vehicle between 35 and 45 days old. Subjects were then allowed to mature to adulthood. At 68–69 days of age, subjects were tested for seizure susceptibility using the pro-convulsant, pentylenetetrazol (PTZ). Subjects received an acute injection of either 35 mg/kg or 50 mg/kg PTZ immediately prior to a 30-min behavioral seizure test. PTZ doses were chosen to produce low-to-moderate levels of seizure activity in control subjects. There were no significant differences between treated and control subjects in: latency to first seizure, mean seizure severity, percentage who displayed any seizure activity, percentage who displayed clonic seizure, or percentage who displayed tonic–clonic seizure. However, CP 55,940-treated subjects had a higher mortality rate compared to controls at both PTZ doses, suggesting that adolescent cannabinoid exposure may increase the lethality of severe seizures experienced in adulthood.

1. Introduction

Chronic use of cannabinoids during adolescence may induce subtle, but profound, detrimental neurobehavioral effects that persist into adulthood. Human neuroimaging and neuropsychological studies have documented a number of deficits and alterations in brain function that appear to be correlated with heavy adolescent cannabis consumption (for reviews, see Batalla et al., 2013; Higuera-Matas et al., 2015; Renard et al., 2014). The preponderance of evidence for cannabinoid-induced deficits comes from non-human studies. For example, adolescent cannabinoid exposure in rats leads to numerous cognitive and behavioral changes in adulthood, including: impaired object memory, increased cocaine and heroin self-administration, altered anxiety, enhanced depressive behavior and anhedonia, and disruption of pre-pulse inhibition (for reviews, see Higuera-Matas et al., 2015; Realini et al., 2009). Many of these effects appear to be sex-dependent (Rubino and Parolaro, 2015). Our own laboratory has shown that chronic treatment with the CB1 receptor agonist, CP 55,940, during adolescence reduces adult female sexual motivation (Chadwick et al., 2011) and negatively impacts female paced mating behavior (Minney and López, 2013). No laboratory has yet examined whether adolescent cannabinoids affect seizure susceptibility in adult rats; this was the primary objective of the current experiment.

Chronic cannabinoid exposure during adolescence may cause abnormal brain development that serves as a risk factor for a variety of psychiatric conditions (Chadwick et al., 2013; Renard et al., 2014). Rodent research has identified the hippocampus (Rubino et al., 2009) and prefrontal cortex (PFC; Ellgren et al., 2008; Rubino et al., 2015) as two potentially vulnerable brain regions. For example, adolescent cannabinoid treatment significantly alters the maturation of glutamate and GABA systems in the hippocampus and cortex (reviewed in Higuera-Matas et al., 2015). Of particular interest, Cass et al. (2014) found that repeated stimulation of the CB1 receptor during early and middle adolescence impaired GABAergic activity within the PFC and elicited an enduring state of neural disinhibition. Abnormal neural excitability could increase susceptibility to certain psychiatric conditions, like schizophrenia (Zamberletti et al., 2014), and, we propose, seizure disorders.

The endogenous cannabinoid system (ECS) is intimately involved in the regulation of brain activity, and normal endocannabinoid function may play an intrinsic protective role in suppressing pathological neuronal excitability (Lutz, 2004; Wallace et al., 2003; Zanettini et al., 2011). There is evidence that the ECS is dysregulated in individuals with epilepsy (Ludányi et al., 2008; Romigi et al., 2010). Therefore, if chronic cannabinoid exposure during adolescence negatively affects the development of the ECS (Rubino et al., 2008, 2015), such exposure may also increase one's susceptibility to neurological and behavioral seizure events later in life.

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It should be noted that there is, currently, enormous interest in the potential therapeutic applications of cannabinoids for the treatment of seizure disorders, such as epilepsy, Dravet syndrome, and spasticity associated with multiple sclerosis. There is growing consensus that certain phytocannabinoids, in particular cannabidiol (CBD), possess significant anticonvulsant efficacy (Devinsky et al., 2014; Hill et al., 2012). Interestingly, CBD does not appear to activate the CB1 receptor. Acute administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) and other CB1 receptor agonists tend to have complex, unpredictable effects on seizures in animal studies. Experimenters have observed both pro- and anticonvulsant effects which may be linked to a variety of factors, including the specific agonist used, dose delivered, seizure model utilized, and species/strain of subject tested. For example, Vilela et al. (2013) found that acute administration of the CB1 agonists, WIN 55,212-2 and ACEA, reduced the threshold for myoclonic seizures and enhanced epileptiform electroencephalogram activity, while URB-597 (a FAAH inhibitor and indirect endocannabinoid agonist) had an anti-convulsive effect in rats.

Cannabis continues to be the most widely used illicit drug throughout the world (United Nations Office on Drugs and Crime, 2013). Furthermore, given the shifting attitude in the United States over the use of medical marijuana, it is likely that more people will transition into chronic, long-term consumption of cannabinoids for treatment of various medical conditions, including seizure disorders. Many of these individuals will be children or adolescents. As such, it is vital that we develop a more comprehensive understanding of how repeated use of cannabinoids during critical development periods can influence brain development and possibly affect subsequent adult psychology and behavior.

The current experiment was designed to test the hypothesis that chronic administration of the CB1 receptor agonist, CP 55,940, during adolescence would subsequently affect seizure susceptibility in adulthood. To assess seizure susceptibility, adult subjects were administered either a sub-convulsive or moderately convulsive dose of pentylenetetrazol (PTZ) and then observed for 30 min. The PTZ model is one of the most commonly used methods to induce seizure in rats and screen for potential anticonvulsants (Velisek, 2006). The procedure is straightforward, and the behaviors produced (freezing, myoclonic twitches, clonic seizure, tonic–clonic seizure) can each be related to seizure phenomena in humans. We predicted that subjects who received daily doses of CP 55,940 during adolescence would, as adults, be more likely to experience severe seizures after acute PTZ administration.

2. Material and methods

2.1. Subjects

Sixty male, Wistar Kyoto rats (Charles River Laboratories, Wilmington, MA) were used in this experiment. Subjects arrived at 27 days of age and were housed individually in plastic cages with corncob bedding within a temperature-controlled (23 ± 2 °C) vivarium. We housed our subjects individually because prior unpublished experiments in our laboratory have shown that male, adolescent rats treated chronically with CP 55,940 can periodically show increased agonistic behavior (often resulting in serious injury) when pair-housed. Therefore, we believe that when using this treatment regimen with males, single-housing is safer and more ethical. However, we also acknowledge that single-housing a social animal during a developmental period characterized by high levels of play behavior may significantly impact neurobehavioral development and alter sensitivity to drugs of abuse (Vanderschuren and Trezza, 2014). The vivarium was programmed with a 12:12 light–dark schedule (lights on 6:00–18:00 h). Food and water were available ad libitum, and all subjects' cages were environmentally enriched with a Nylabone® and a short length of PVC pipe. All experimental protocols were approved by the campus Institutional Animal Care and Use Committee (IACUC) in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Procedure

Subjects were handled daily for 7 days prior to initiation of experimental procedures. Daily treatment with either cannabinoid or vehicle occurred for 11 days, between post-natal day (PND) 35 and PND 45. Subjects were randomly assigned to either the experimental group or the control group. Experimental subjects received a single daily dose of 0.4 mg/kg CP 55,940 (Sigma-Aldrich, St. Louis, MO) dissolved in a vehicle of physiological saline, Kolliphor, and ethanol (18:1:1). All injections were intraperitoneal (IP) in a volume of 1 mg/ml, administered 1–2 h into the subject's activity cycle (~20:00 h). Control subjects were administered daily injections of vehicle in the same volume. We have used this treatment window and dosage regimen in previous experiments (Chadwick et al., 2011; Minney and López, 2013); it is not associated with any immediate adverse health effects but can induce persistent neurobehavioral changes into adulthood.

Between PND 46 and 67, experimenters handled and weighed subjects 2–3 times per week, but otherwise allowed the subjects to mature without any experimental manipulations. On PND 68, subjects were habituated to the seizure testing room and observation chambers for 15 min each.

On PND 69 and 70, subjects were tested for seizure susceptibility. Thirty-two subjects were tested the first day and twenty-eight on the subsequent day. Seizure testing took place in a separate room under normal light conditions, beginning around 10:00 h. To induce seizure, subjects were administered a single IP dose of the proconvulsant compound, pentylenetetrazol (PTZ). Two different doses of PTZ were used. Half of the subjects in each adolescent treatment condition were given 35 mg/kg PTZ and the other half received 50 mg/kg. PTZ was dissolved in a vehicle solution of physiological saline, and injection volumes were 1 ml/kg.

Given our hypothesis that adolescent cannabinoid treatment would increase seizure susceptibility, we chose PTZ doses that would allow us to see an increase in seizure activity. Numerous studies using adult male Wistar rats (e.g., Atapour et al., 2000; Silva et al., 2013) have demonstrated that 35 mg/kg is a sub-convulsant dose that does not typically induce seizure activity beyond myoclonic twitches. 50 mg/kg PTZ is considered an intermediate dose that reliably induces clonic seizure components (Cortez and Snead, 2006) and allows for observation of either increased or reduced seizure severity following experimental treatment.

Four subjects, one from each treatment group, were tested simultaneously. Immediately after PTZ administration, each subject was transferred into a transparent plastic cage (27 × 16.5 × 12 cm) for observation. A wooden divider was placed between cages so that subjects being tested simultaneously were not visible to each other. The seizure test lasted for 30 min. Tests were video-recorded using a tripod-mounted digital camera (NTSE SONY XC-EI50). Video was digitized at 60 Hz in 640 × 840 resolution onto a Macintosh computer. Video was processed using unpublished software (RatCam) using a frame-by-frame accurate timecode.

Seizure videos were subsequently coded, independently, by two raters blind to the treatment condition of each subject. Individual seizures were scored on a 5-point severity scale appropriate for generalized seizures with forebrain origin (Pohl and Mares, 1987; Veliskova, 2006): 1, isolated myoclonic jerks; 2, atypical clonic seizure; 3, fully developed bilateral forelimb clonus; 3.5, forelimb clonus with tonic component and body twist; 4, tonic–clonic seizure with suppressed tonic phase, loss of righting; 5, fully developed tonic–clonic seizure with loss of righting. For each subject, latency to first sign of seizure and maximum seizure severity achieved were coded. The following group variables were then calculated: 1) mean latency to first seizure, 2) mean maximum seizure severity, 3) percentage of subjects that displayed