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DELTA-9-TETRAHYDROCANNABINOL (THC) AFFECTS FORELIMB MOTOR MAP EXPRESSION BUT HAS LITTLE EFFECT ON SKILLED AND UNSKILLED BEHAVIOR

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Abstract—It has previously been shown in rats that acute administration of delta-9-tetrahydrocannabinol (THC) exerts a dose-dependent effect on simple locomotor activity, with low doses of THC causing hyper-locomotion and high doses causing hypo-locomotion. However the effect of acute THC administration on cortical movement representations (motor maps) and skilled learned movements is completely unknown. It is important to determine the effects of THC on motor maps and skilled learned behaviors because behaviors like driving place people at a heightened risk. Three doses of THC were used in the current study: 0.2 mg/kg, 1.0 mg/kg and 2.5 mg/kg representing the approximate range of the low to high levels of available THC one would consume from recreational use of cannabis. Acute peripheral administration of THC to drug naïve rats resulted in dose-dependent alterations in motor map expression using high resolution short duration intracortical microstimulation (SD–ICMS). THC at 0.2 mg/kg decreased movement thresholds and increased motor map size, while 1.0 mg/kg had the opposite effect, and 2.5 mg/kg had an even more dramatic effect. Deriving complex movement maps using long duration (LD)–ICMS at 1.0 mg/kg resulted in fewer complex movements. Dosages of 1.0 mg/kg and 2.5 mg/kg THC reduced the number of reach attempts but did not affect percentage of success or the kinetics of reaching on the single pellet skilled reaching task. Rats that received 2.5 mg/kg THC did show an increase in latency of forelimb removal on the bar task, while dose-dependent effects of THC on unskilled locomotor activity using the rotorod and horizontal ladder tasks were not observed. Rats may be employing compensatory strategies after receiving THC, which may account for the robust changes in motor map expression but moderate effects on behavior.

Key words: intracortical microstimulation, tetrahydrocannabinol, skilled reaching, locomotor activity, motor maps, rats.

INTRODUCTION

While the hemp plants *Cannabis sativa* and *indica* (commonly referred to as cannabis) have been used recreationally and medicinally for thousands of years (Felder and Glass, 1998), their use in Western nations is on the rise. The report from the Substance Abuse & Mental Health Services Administration in 2014 indicates that the rate of current illicit drug use, of which 80% of those surveyed reported using cannabis, increased from 7.9% in 2002 to 9.4% in 2013. The percentage of people that reported "using cannabis in the past month" also increased from 5.8% in 2002 to 7.5% in 2013. Potential locomotor impairments induced by cannabis have come to the attention of researchers due to the inherent safety issues while driving under the influence (Shi et al., 2005; Ramaekers et al., 2006; Smirnov and Kiyatkin, 2008; Sewell et al., 2009). Moreover, with the legalization of cannabis in certain states within the United States (Hall, 2015a) and the development of a cannabis breathalyzer it has become increasingly important to study the dosage effects of the psychoactive ingredients in cannabis on brain and locomotor behavior, particularly skilled movements.

The main psychoactive agent in cannabis is delta-9-tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964; Mechoulam et al., 1970), which binds to two G-protein-coupled cannabinoid receptors termed CB1 and CB2 (Harris et al., 1978; Devane et al., 1988; Matsuda et al., 1990; Herkenham et al., 1991; Munro et al., 1993). CB1 receptors are highly expressed in motor structures including the cortex, basal ganglia, and cerebellum, and are found at axon terminals of glutamatergic pyramidal neurons and GABAergic interneurons (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998; Castillo et al., 2012), while CB2 receptors have limited expression in the brain (Lynn and Herkenham, 1994; Van Sickle et al., 2005; Castillo

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Abbreviations: ANOVA, analysis of variance; CB, cannabinoid; THC, delta-9-tetrahydrocannabinol; DMSO, dimethyl sulfoxide; i.p., intraperitoneal; LD–ICMS, long duration–intracortical microstimulation; LE, Long Evans; RPM, rotations per minute; SD–ICMS, short duration–intracortical microstimulation.

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et al., 2012). Activation of CB1 receptors leads to attenuation of neurotransmitter release via hyperpolarization and prevention of calcium entry into the pre-synaptic cell, thereby blunting both inhibitory and excitatory synaptic transmission (Howlett and Mukhopadhyay, 2000; Sharkey and Pittman, 2005). While there has been a small amount of research investigating the effects of THC on motor function, the effects of THC on motor map expression have yet to be explored.

Motor maps are the topographical representation of movement in the brain. Forelimb motor maps are routinely produced using two methods with different durations of intracortical microstimulation (ICMS). Short duration (SD)–ICMS produces simple, single joint movements such as digit, wrist, elbow and shoulder, while long duration (LD)–ICMS produces complex, multi-joint movements such as elevate, advance, grasp and retract (Ramanathan et al., 2006; Harrison et al., 2012; Bonazzi et al., 2013; Brown and Teskey, 2014). Forelimb motor maps produced by SD–ICMS have been used as an indication of the balance between excitation and inhibition in motor cortex (Teskey et al., 2007; Henderson et al., 2012; Hussin et al., 2015) and show a number of plastic effects in response to differential experience (Kleim et al., 1998; Young et al., 2012) and pathological conditions (van Rooyen et al., 2006; Brown et al., 2009, 2011). Manipulations that shift the balance toward more inhibition increase movement thresholds and decrease motor map size, while manipulations that shift the balance toward excitation have the opposite effect (Monfils et al., 2004; Henderson et al., 2011; Young et al., 2011, 2012; Scullion et al., 2013). LD–ICMS is used to examine the expression of complex movements (Graziano et al., 2002; Brown and Teskey, 2014). Both types of movement representations can be altered by the type and quantity of neurotransmitter present in the motor cortex (Conner et al., 2003; Metz et al., 2004; Brown et al., 2009, 2011; Ramanathan et al., 2009; Viaro et al., 2011; Scullion et al., 2013) with a particular emphasis on glutamate and GABA (Hussin et al., 2015).

While there is some evidence to suggest that THC has a dose-dependent effect on unskilled locomotor behavior (Wiley et al., 2007; Sañudo-Peña et al., 2011; Katsidoni et al., 2013), the effect of THC on motor maps or skilled reaching behavior has not been assessed at any dose. We employed the single pellet skilled reaching task, which requires rats to produce a complex multi-joint coordinated behavior and gives both end-points (reach attempts and success) and reaching motion, which is parsed into 10 discrete subcomponents (Whishaw et al., 2003; Monfils and Teskey, 2004; Henry et al., 2008). Studies that have altered the cholinergic, serotonergic and dopaminergic systems or have created brain lesions or seizures have shown rats exhibit a decrease in reaching performance and alterations in motor map expression (Whishaw et al., 1992, 1993, 2007; Gharbawie and Whishaw, 2003; Kleim et al., 2003; Gharbawie et al., 2005; Henry et al., 2008; Conner et al., 2010; Flynn et al., 2010; Boychuk et al., 2011; Brown et al., 2011; Scullion et al., 2013). Therefore, the single pellet skilled reaching task has previously been used as an indicator of brain function and is sensitive to neurotransmitter system manipulations. The purpose of this study was to determine, for the first time, the skilled and unskilled locomotor impairments and motor map expression at three ecologically valid and acute doses of THC. To test the hypothesis that THC dose dependently affects motor map expression we conducted SD–ICMS map–remap studies using low, medium and high dose of THC. Next, having shown an inhibitory effect of 1.0 mg/kg THC on SD–ICMS-derived motor maps, we determined the effect of 1.0 mg/kg of THC on complex movements using LD–ICMS. In separate groups of rats we conducted several behavioral tests to examine the effects of THC on both skilled behavior using the single-pellet reaching task and unskilled behavior using a small battery of 3 locomotion-based assays.

EXPERIMENTAL PROCEDURES

Rodents

Adult male Long Evans (LE) rats (n = 61) were used in this study. All rats were obtained from Charles River (Quebec, Canada; North Carolina, USA). All experiments were approved by the Health Sciences Animal Care Committee at the University of Calgary. Rats were maintained and handled in accordance to the Canadian Council for Animal Care guidelines. Rats were maintained on a 12-h light/dark cycle with lights on at 07:00 h and pair housed (except for the reach training study where they were housed individually) in clear plastic cages in a colony room. Rats had free access to food and water except rats in the reaching experiment where weight and behavioral signs of hunger (moving too quickly during the reach training sessions/excessive reach attempts) were monitored daily to determine the amount of food given per day. All experiments were performed during the light phase.

Drugs

THC was obtained through Sigma Aldrich (Oakville, ON, CAN) and was dissolved in 100% dimethyl sulfoxide (DMSO). Three different concentrations (0.2 mg/kg; 1 mg/kg; 2.5 mg/kg) of Δ9 THC were chosen since hyper- and hypo-locomotions have been shown to occur at these doses (Sañudo-Peña et al., 2011; Katsidoni et al., 2013). We chose not to use any higher doses as we did not want to induce catalepsy (Sañudo-Peña et al., 2011). THC was acutely administered via intraperitoneal (i.p.) injection with an injection volume of 1 ml/kg for all experiments. The THC dosages used for SD ICMS were 0.2 mg/kg, 1.0 mg/kg and 2.5 mg/kg while LD ICMS used 1.0 mg/kg. A dosage of 1.0 mg/kg and 2.5 mg/kg of THC was used in the single pellet skilled reaching task. The unskilled motor tasks were performed using 0.2 mg/kg, 1.0 mg/kg and 2.5 mg/kg.

SD ICMS

Standard high-resolution intracortical microstimulation (ICMS) techniques were used to produce detailed