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 rotordor 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. © 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

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, 1999), 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 et al., 2008).
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, Canada) 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
threshold maps of forelimb regions of the motor cortex (Nudo et al., 1990; Kleim et al., 1998; Young et al., 2011). Twenty-four hours prior to surgery, rats were food deprived. Rats were initially anesthetized by an i.p. injection of ketamine (100 mg/kg) and xylazine (5 mg/kg). Supplemental injections of either ketamine (25 mg/kg) or a cocktail of ketamine (17 mg/kg) and xylazine (2 mg/kg) were given as needed to maintain a light anesthetic level. Level of anesthesia was monitored by assessment of breathing rate, whisker movements, and withdrawal reflex from a light pinch to the foot.

A craniotomy was performed to expose the motor cortex of the left or right hemisphere. From the bregma, the craniotomy extended approximately 4 mm anterior and 3 mm posterior, and extended approximately 5 mm lateral from the midline in rats. The cisterna magna was punctured with an 18-gauge needle to reduce brain swelling, and the dura mater was carefully removed from the neocortex. Silicon liquid warmed to body temperature (37–38°C) from the neocortex. Silicon liquid was injected i.p. and 15 min later the motor cortex was re-mapped. Following the baseline ICMS mapping either ICMS was first performed to derive a baseline motor movement threshold as an indicator of anesthesia level. Level of anesthesia was monitored by assessment of breathing rate, whisker movements, and withdrawal reflex from a light pinch to the foot.

LD ICMS

Induction and maintenance of anesthesia as well as the craniotomy procedure were completed in the same manner as SD ICMS. An injection of DMSO (vehicle) was given 15 min prior to the first electrode penetration. LD–ICMS methodology was used according to Brown and Teskey (2014). Glass insulated platinum (80%), iridium (20%) microelectrodes (FHC, Inc., Bowdoin, ME, USA) with impedance values of 0.3–0.5 MΩ were used. The microelectrode was lowered to a depth of 1550 μm and stimulation consisted of 500 ms trains of 200-μs biphasic pulses, delivered at a frequency of 333 Hz and an intensity of 100 μA. Biphasic pulses were chosen to avoid tissue damage (Tehovnik, 1996; Graziano et al., 2002). Each penetration site was stimulated at a frequency of 0.2 Hz to a maximum of six times to ensure consistency of evoked responses and to prevent the spread of neuronal activation (Nudo et al., 1990; Brown and Teskey, 2014). The forelimb was lightly adjusted to baseline position after each stimulation. The penetration site was considered non-responsive if a movement was not evoked at 100 μA on more than half of the delivered stimulation trains. The first penetration site occurred at 1 mm anterior and 2 mm lateral of the bregma. Each successive penetration site moved in the parasagittal direction until a non-forelimb (vibrissae, neck, jaw, trunk, hindlimb, tail) or a non-responsive occurred. The map was considered complete once all forelimb points were bordered by non-forelimb or non-responsive sites.

Evoked movements were classified as simple or complex. Simple movements involved a single forelimb joint and were digit flexion, elbow extension, elbow flexion, wrist extension and supination of the forelimb. Complex movements involved multiple forelimb joints and included elevate (flexion of the elbow followed by extension of the wrist), advance (forward displacement of the elbow and shoulder with wrist extension and hand opening), grasp (flexion of the wrist and simultaneous digit contraction and hand closure) as well as retract (caudal displacement of the elbow and shoulder).

Once a baseline (vehicle) map had been derived, 1.0 mg/kg THC (n = 6) was injected i.p. and 15 min later the motor cortex was re-mapped. Motor maps were analyzed for movement representation topography. A dose of 1.0 mg/kg THC was chosen for this experiment as it was shown to have an inhibitory effect on SD ICMS maps.

Single pellet skilled reaching task

To determine the effect of acute THC on performing a task involving skilled use of the forelimb, we trained rats on the single pellet reaching task (Whishaw et al., 2003). In this task rats must learn to reach for a sugar pellet on a shelf through a slit in the apparatus. Banana-flavored sucrose pellets (90 mg of Rodent Chow food pellets; Bioserve, Flemington, NJ, USA) were placed in the home cage and rats were food restricted to 85–90% of free-feeding levels prior to pre-training. This was done to familiarize the rats to the sugar pellets and ensure motivation for reaching. Pre-training was conducted on
adult male LE rats \(n = 5\) DMSO, \(n = 6\) 1.0 mg/kg THC, \(n = 6\) 2.5 mg/kg THC) to determine the handedness of the rat. Rats then underwent one, 15-min reach training session for 17 consecutive days. We measured the number of reach attempts and percent success on each training session. If rats did not reach an average of 50% success across days 13–15 they were eliminated from the study. On day 16 rats received an i.p. injection of 1.0 mg/kg THC, 2.5 mg/kg THC or vehicle (DMSO) 15 min prior to the start of the reaching session. The reach training session on day 17 occurred 24 h post injection from day 16. On days 15–17 the reaching session was video-taped for future analysis of the 10 discrete subcomponents that were assessed according to an ordinal rating scale. (1) Digits to the midline: the reaching limb is lifted from the floor so that the tips of the digits are aligned with the midline of the body; (2) Digits semi-flexed: As the limb is lifted, the digits are maintained in a semi-flexed position; (3) Elbow to midline: the elbow is adducted to the midline while the tips of the digits retain their alignment with the midline of the body; (4) Advance: The limb is advanced directly through the slot toward the food pellet; (5) Digits extend: the digits extend during the advance so that the digit tips are pointing toward the target; (6) Arpeggio: While the forelimb is over the target, the hand pronates from digit 5 (the outer digit) through to digit 2 while the hand simultaneously opens; (7) Grasp: The digits flex with the hand closing over the pellet, and the wrist is extends slightly; (8) Supination I: As the limb is withdrawn, the hand supinates by nearly 90° to allow withdrawal through the slot; (9) Supination II: Once withdrawn from the slot, the hand further supinates by nearly 45° to place the food in the mouth; (10) Release: The hand contacts the mouth and opens to release the food. Movements that appeared normal were given a score of 0, ambiguous movements a score of 0.5, impaired but recognizable movements a score of 1, and absent or unrecognizable movements a score of 2. All apparatus, training method, and analysis have previously been described (Whishaw et al., 2003; Brown and Teskey, 2014).

Unskilled motor tasks

All rats were habituated to handling prior to performing the unskilled motor tasks. Each unskilled motor task was performed once by the rats in the following order: Bar task, rotorod, and horizontal ladder rung walking.

Bar task. We used the bar task to measure response time. A metal bar 4.76 mm in diameter was placed through two holes that were drilled into a standard home cage at a height of 8.89 cm. Rats in their home cage had their forepaws placed on the metal bar and hindpaws remained on the bottom surface. We digitally recorded this task for an offline analysis of the latency it took for the rats to remove both forepaws off the bar.

Rotorod. An accelerating protocol ranging from four to 40 rotations per minute (RPM) with a trial length of five minutes was used. Rats were placed on the rota rod (Panlab; Harvard Apparatus) with an initial RPM of four. We recorded the latency to fall and the RPM at which the rat fell.

Horizontal ladder rung walking

The apparatus was comprised of 20.32-cm-high walls made of clear Plexiglas which were 1 m in length and metal rungs that were 3 mm in diameter, which created a floor. The metal rungs were irregularly spaced such that no more than 2 rungs were removed in sequence. The width of the apparatus was variable such that it was always slightly wider than the rat, in order to prevent the rats from turning around. A bright light was placed at one end of the ladder while a home cage was placed at the end in order to provide incentive to the rat to cross the ladder. We digitally recorded the crossing of the ladder for offline analysis of limb placement using VLC media player (http://www.videolan.org/vlc/index.html).

Horizontal ladder rung walking

We used a modified version of Metz and Whishaw (2002, 2009) scoring system for each paw placement; where 0 represents a perfect placement, 1 a slight slip, 2 a deep slip and 3 a fall or complete miss. The first and last step cycle were not analyzed. We also analyzed the time it took to cross the horizontal ladder.

Statistics

SD ICMS map–re-map data were analyzed using paired samples t-tests, while LD ICMS map–re-map data were analyzed using the Wilcoxon signed ranks test. Number of reach attempts, percentage success and time to execute the reaching motion were analyzed using a repeated measures analysis of variance (ANOVA) with Bonferroni post hoc comparisons. The 10 subcomponents of reaching were analyzed using the Friedman test with the Wilcoxon signed ranks test post hoc comparisons using Bonferroni adjusted p values for multiple comparisons. Data from the rotorod, bar task, as well as time to cross from the rung walking task were analyzed using an ANOVA with Tukey post hoc comparisons using Bonferroni adjusted values. Data were analyzed using a non-parametric ANOVA. An alpha value of 0.05 was used in all experiments and all statistical analyses were conducted using Statistical Package for the Social Sciences (IBM SPSS). Data are presented as mean ± SEM.

RESULTS

SD ICMS and THC 0.2, 1.0, 2.5 mg/kg map-remaps

An i.p. injection of DMSO (vehicle) did not have a significant \(t(4) = 0.86, p = 0.44\) effect on movement thresholds compared to pre DMSO thresholds. DMSO also did not significantly \(t(4) = 1.77, p = 0.15\) affect forelimb motor map size compared to pre DMSO map size.

THC was used at various doses to assess for effects on motor map expression. 0.2 mg/kg of THC caused a significant \(t(3) = 6.10, p = 0.01\) decrease in movement thresholds and a significant \(t(3) = −5.44, p = 0.01\) increase in forelimb motor map size compared to pre-drug values (Figs. 1 and 2).
The effects of 1.0 mg/kg and 2.5 mg/kg THC were opposite to that of 0.2 mg/kg THC and 1.0 mg/kg. 1.0 mg/kg THC caused a significant \( t(3) = -4.48, p = 0.02 \) increase in movement thresholds and a significant \( t(3) = 5.31, p = 0.01 \) decrease in forelimb motor map size compared to pre-drug values. 2.5 mg/kg also caused a significant \( t(4) = -8.36, p = 0.001 \) increase in movement thresholds and a significant \( t(4) = 5.52, p = 0.005 \) decrease in forelimb motor map size compared to pre 2.5 mg/kg THC values (Figs. 1 and 2). The reduction in map size was significantly \( t(7) = 3.34, p = 0.01 \) greater in rats that received 2.5 mg/kg compared to rats that received 1.0 mg/kg THC.

No significant differences were found in the amount of ketamine \( F_{(3,14)} = 1.91, p = 0.18 \) or xylazine \( F_{(3,14)} = 1.35, p = 0.29 \) as a function of body weight and duration of surgery between rats that received DMSO and rats that received the various doses of THC. This indicates that changes seen in movement threshold and map size were not a function of anesthesia depth.

THC has a dose-dependent effect on motor map expression. 0.2 mg/kg decreased forelimb movement thresholds and increased motor map size, while 1.0 mg/kg and 2.5 mg/kg increased movement thresholds and decreased motor map size. Previous research has shown that disruption in forelimb motor map expression...
can affect skilled forelimb movements (Kleim et al., 2003; Flynn et al., 2010; Brown et al., 2011; Henderson et al., 2012; Scullion et al., 2013). Therefore we next examined how a dose of 1.0 mg/kg THC affects the expression of complex movement representations using LD ICMS.

LD ICMS and 1.0 mg/kg THC map-remap

There was a significant ($Z = -2.03$, $p = 0.04$) decrease in the number of complex multi-joint movements in the THC post-map (12.50 ± 2.33) compared to the DMSO pre-map (17.67 ± 3.81; Fig. 3). Since THC decreased the number of complex multi joint movements in LD ICMS motor maps we then determined the effects of THC on skilled reaching.

THC and skilled reaching

DMSO ($n = 5$) did not have a significant effect on the number of reach attempts ($F_{(2,8)} = 0.99$, $p = 0.41$), or the percentage of success ($F_{(2,8)} = 0.42$, $p = 0.67$). 1.0 mg/kg of THC ($n = 6$) significantly ($F_{(2,10)} = 18.04$, $p = 0.0001$) reduced the number of reach attempts compared to pre THC ($p = 0.02$) and post THC ($p = 0.02$). However, 1.0 mg/kg THC did not have a significant ($F_{(2,10)} = 1.91$, $p = 0.20$) effect on percentage success (Fig. 4). 2.5 mg/kg of THC ($n = 6$) significantly ($F_{(2,10)} = 6.54$, $p = 0.01$) reduced the number of reach attempts compared to pre-THC ($p = 0.04$) but did not significantly alter percentage of success ($F_{(2,10)} = 0.44$, $p = 0.67$; Fig. 4).

Since it was found that rats administered THC performed less reach attempts we then examined if the reason for the reduction in reach attempts was due to a slower execution of the reaching motion. 1.0 mg/kg ($F_{(2,8)} = 0.55$, $p = 0.51$; $n = 4$) and 2.5 mg/kg ($F_{(1,4)} = 0.34$, $p = 0.59$; $n = 5$) THC did not have a significant effect on the time it took to execute the reaching motion (Fig. 4).

A frame-by-frame analysis of forelimb movements revealed there were no significant differences in the mean error score for the ten sub components of reaching in the DMSO or THC groups (Fig. 4).

1.0 mg/kg and 2.5 mg/kg reduced the number of reach attempts in a training session.

This reduction in attempts was not due to the execution of the reach taking a longer period of time. 1.0 mg/kg and 2.5 mg/kg did not affect the success rate and did not impair the 10 subcomponents of reaching. We then determined if THC affected unskilled motor...
tasks using bar, rotorod, horizontal ladder run walking, and open-field tasks.

THC and unskilled motor tasks

Bar task. One rat from the DMSO group was excluded from analysis on the bar task as the latency to remove both forepaws (2.55 s) from the bar was six standard deviations away from the DMSO group mean. There was a significant (\(F(3,27) = 2.838, p = 0.05\); DMSO: \(n = 7\); 0.2 mg/kg: \(n = 7\); 1.0 mg/kg: \(n = 7\); 2.5 mg/kg: \(n = 7\)) group effect on the latency to remove both forepaws from the bar. Post-hoc analysis revealed rats that received 2.5 mg/kg THC held onto the bar longer than rats that received DMSO (\(p < 0.05\); Fig. 5).

Rotorod. One rat from the DMSO group was excluded from analysis on the rotorod as the RPM (29) and latency to fall (204 s) for this rat were 13 and 14 standard deviations respectively, away from the DMSO group mean (Fig. 5). There was no significant difference in the latency to fall (\(F(3,27) = 1.39, p = 0.26\) or the RPM at which the rat fell (\(F(3,27) = 0.99, p = 0.41\); DMSO: \(n = 7\); 0.2 mg/kg: \(n = 7\); 1.0 mg/kg: \(n = 7\); 2.5 mg/kg: \(n = 7\)) on the rotorod test.
Horizontal ladder rung walking task. No significant differences were found in the mean error scores for the right forepaw ($\chi^{(3)} = 1.95, p = 0.58$), left forepaw ($\chi^{(3)} = 5.05, p = 0.17$), right hindlimb ($\chi^{(3)} = 2.53, p = 0.47$), and the left hindlimb ($\chi^{(3)} = 2.32, p = 0.51$) across the four groups (DMSO, 0.2, 1.0, 2.5 mg/kg THC). THC did not cause a significant ($F_{(3,28)} = 0.98, p = 0.42$; DMSO: $n = 8$; 0.2 mg/kg: $n = 8$; 1.0 mg/kg: $n = 8$; 2.5 mg/kg: $n = 8$) difference in the time it took to cross the horizontal ladder across groups (Fig. 5).

### DISCUSSION

This is the first study to demonstrate a dose-dependent effect of THC on the expression of forelimb motor maps. We observed that low-dose (0.2 mg/kg) THC decreased movement thresholds and increased motor map size, while higher dosages (1.0 mg/kg and 2.5 mg/kg) elicited the opposite effect, with 2.5 mg/kg having the greatest effect. A dose of 1.0 mg/kg THC also reduced the number of complex movements elicited by LD–ICMS, complementing the results found for SD–ICMS. Rats that received 1.0 mg/kg THC or 2.5 mg/kg THC had a significant reduction in the number of reach attempts, but there were no significant differences in the percentage of success or the 10 subcomponents of reaching in the skilled single pellet reaching task. We also found rats that received 2.5 mg/kg THC took longer to remove their forepaws from the bar compared to the vehicle, 0.2 mg/kg and 1.0 mg/kg groups, indicating hypo-locomotion.

The results from the ICMS experiments are consistent with previous studies showing that low doses of THC or CB1 receptor agonists are stimulatory, while high doses are inhibitory on unskilled locomotor behavior (Sañudo-Peña et al., 2011; Katsidoni et al., 2013), transcallosal...
evoked potentials (Turkanis and Karler, 1981), neurotransmitter release within the hippocampus (Tzavara et al., 2003), anxiety (Ruehle et al., 2012) and feeding (Bellochio et al., 2010). While activation of CB1 receptors typically leads to reduction of neurotransmitter release, thus reducing activation of the post-synaptic neuron (Howlett and Mukhopadhyay, 2000; Sharkey and Pittman, 2005) the most parsimonious explanation for the biphasic effect of THC is that low doses of THC preferentially affect CB1 receptors located on GABAergic interneurons due to higher CB1R expression, while higher doses of THC affect CB1 receptors located on pyramidal neurons (Mackie, 2007). Activation of CB1 receptors on interneurons would result in a release of inhibition of pyramidal neurons, which could conceivably produce lower movement thresholds and larger motor maps due to an increase in the balance of cortical excitation compared to inhibition. Activation of CB1 receptors on pyramidal neurons could shift the balance in favor of inhibition with less glutamate released by the pyramidal neurons resulting in higher movement thresholds and smaller motor maps.

In the current study we did not observe the biphasic effects of THC on locomotor tasks as previously reported by Sañudo-Peña et al. (2011) and Katsidoni et al. (2013). The difference in our findings from previous studies maybe due to the strain difference in the rats. Previous studies used Sprague–Dawley rats while our study employed Long-Evans rats. Behavioral differences between Long-Evans and Sprague–Dawley in motor behavior, the stress response, as well as learning and memory have been reported (Bielsew et al., 2002; Harker and Whishaw, 2002; Whishaw et al., 2003). Rats are usually pre-trained in the rotorod and horizontal ladder rung walking task (Metz and Whishaw, 2002; Buitrago et al., 2004). Pre-training allows rats to become proficient at the task and therefore could alter the effect of THC on the task. We chose not to do this as we wanted the purest measure of unskilled motor performance under the influence of THC. Perhaps, if the rats had been pre-trained on the rotorod and rung walking task, we would have seen the biphasic effect of THC on locomotion previously described by other researchers (Sañudo-Peña et al., 2011; Katsidoni et al., 2013). A further consideration is the order in which we conducted the unskilled tasks. Previous research has demonstrated that order effects occur in test batteries and that performance on tasks are altered if the task is performed in a battery versus if there is only one task to perform (McIlwain et al., 2001; Blokland et al., 2012). However, to the best of our knowledge, the order in which we had the rats perform the task went from least stressful to most stressful to minimize the possibility of testing history influencing performance. The dissociation between forelimb motor map expression and some of the freely moving behaviors is what is particularly interesting about the effect of THC. There is not a perfect relationship between forelimb motor map expression and something as complex as motor behavior in a freely moving animal nor should one expect there to be. This is likely true for two main reasons; (1) motor map expression is a relatively simple assessment of highly complex neuronal networks compared to ongoing multifaceted behavior in freely moving animals and (2) other descending motor inputs such as thalamic, rubrospinal, cerebellar, as well as spinal cord circuitry itself all play a role in ongoing behavior.

Rats that received 2.5 mg/kg took longer to remove their forepaws from the bar during the bar task than rats that received DMSO. This effect does not seem to reflect a measure of catalepsy, as the latency to remove both forepaws from the bar in the 2.5 mg/kg group was approximately 3 s and the available literature uses a latency of 10 s or greater as the operational definition of catalepsy (Sanberg et al., 1988). The increase in latency to remove forepaws from the bar may be an indicator of hypo-locomotion and an increase in reaction time. Human studies have found that THC administration results in an increase in reaction time during virtual driving tasks (Hall, 2015b). Human research on driving performance after cannabis consumption that used simulated and on-road testing conditions have found deficits in tracking, reaction time, concentration, short-term memory and divided attention (Beirness and Porath-Waller, 2015). Other research has shown that cannabis users are aware of their impairments and therefore use compensatory mechanisms in an effort to reduce the deficits brought on by cannabis use. Some of these compensatory mechanisms involve driving slower, and increasing the distance between vehicles (Sewell et al., 2009). However, even with these compensatory mechanisms in place, people under the influence of cannabis are twice as likely to be involved in a motor vehicle accident than people who have not used cannabis (Beirness and Porath-Waller, 2015).

The administration of THC prior to the single pellet skilled reaching task resulted in a significant decrease in the number of reach attempts made during the 15-min training session. While all rats were food restricted to 85–90% free feeding levels to ensure motivation for food our result could be due to a lack of motivation for food as high doses of CB1 agonists are anorexic (Bellochio et al., 2010). Another possibility is that the decrease in reach attempts was caused by rats slowly executing the act of reaching compared to baseline. However this was not the case as there was no significant difference in the amount of time it took a rat to complete the reaching action during THC administration compared to baseline. Human research examining effects of THC intoxication has shown that THC reduces a person’s attention, and concentration among other cognitive tasks (Kelly et al., 2004; Ramaekers et al., 2009; Desrosiers et al., 2015; Hall, 2015b). Thus it is possible rats that received 1.0 mg/kg and 2.5 mg/kg THC prior to the reaching task had reduced attentional or motivational states to keep the number of reach attempts comparable to that seen before THC administration.

Motor maps exhibit experience dependent plasticity. It has been previously demonstrated that disruptions to neurotransmitter systems result in alterations to motor map expression (maps usually become smaller) and that these rats are also impaired at performing the single pellet skilled reaching task (Conner et al., 2003; Ramanathan et al., 2009; Brown et al., 2011; Scullion et al., 2013). Changes in motor map expression caused...
by neuropathophysiological conditions such as stroke and seizures (Kleim et al., 2003; Henry et al., 2008), as well as techniques that increase motor cortex excitability (Teskey et al., 2008; Henderson et al., 2012) also produce deficits in fine motor control. Therefore it was surprising we did not find a significant increase in the mean error scores on the 10 subcomponents of forelimb movements, or percentage of success during skilled reaching behavior for rats that received THC compared to rats that received DMSO alone. The fact that there were clear alterations in motor map expression without alterations in reaching behavior could be due to reaching behavior isn’t strictly controlled by the motor cortex but also involves other motor structures allowing for compensation. It is also possible that because ICMS is conducted in the anesthetized state that an interaction between the ketamine/xylazine and THC had an effect not observed in the unanesthetized freely behaving rats.

CONCLUSION

These data indicate that THC dose dependently affects motor map expression in rats such that low doses of THC decreases movement thresholds and increases forelimb map size, while high doses of THC have the opposite effect. Behaviorally higher doses of THC caused a decrease in reach attempts and increased grasp latency in the bar task. Based off of a cannabis cigarette weight of approximately 1 g containing 4–6% THC and given that only 10–25% of available THC enters systemic circulation when inhaled, it takes 0.2–4.4 mg of THC to elicit pharmacological effects in humans (Adams and Martin, 1996; Iversen, 2008). Therefore, while it is difficult to directly compare the dosages used in this study to those that are found in smoked cannabis, the dosages we chose are similar to levels that would be seen following recreational consumption of cannabis. This indicates that it is possible that smoking cannabis cigarette may alter motor cortex excitability but with little effect on motor behavior.

CONFLICTS OF INTEREST

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