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Research report

Acute Δ-9-tetrahydrocannabinol administration in female rats attenuates immediate responses following losses but not multi-trial reinforcement learning from wins

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ABSTRACT

Δ-9-Tetrahydrocannabinol (THC) is the main psychoactive component of marijuana and has potent effects on decision-making, including a proposed reduction in cognitive flexibility. We demonstrate here that acute THC administration differentially affects some of the processes that contribute to cognitive flexibility. Specifically, THC reduces lose-shift responding in which female rats tend to immediately shift choice responses away from options that result in reward omission on the previous trial. THC, however, did not impair the ability of rats to flexibly bias responses toward feeders with higher probability of reward in a reversal task. This response adaptation developed over several trials, suggesting that THC did not impair slower forms of reinforcement learning needed to choose among options with unequal utility. This dissociation of THC's effects on innate/rapid and learned/gradual decision-making processes was unexpected, but is supported by emerging evidence that lose-shift responding is mediated by neural mechanisms distinct from those involved in other forms of reinforcement learning. The present data suggest that, at least in some tasks, the apparent reductions in cognitive flexibility by THC may be explained by the immediate effects on loss sensitivity, rather than impairments of all processes used for choice adaptation.

1. Introduction

THC and other components in marijuana are among the most commonly used illicit drugs in the world. A recent report [1] indicates over 181 million users world-wide, while an estimated 13.1 million people exhibit cannabis dependence globally [2]. Marijuana usage is increasing in many countries, [3] and chronic use appears to be a risk factor for several mental illnesses including psychosis [4] and depression [5]. Moreover, chronic use is also linked to reduced performance on decision-making tasks [6,7]. It is therefore important to better understand how THC affects the neural processes involved in cognition and decision-making. Previous studies have indicated deficits in executive function, verbal and visual memory, and visuoperception following chronic cannabis use in humans [8]. Rodent models have likewise shown impairments in working memory [9,10] and spatial memory [11] following acute THC administration. Furthermore, acute THC causes impairments in reversal learning in macaques [12], as well as reversal learning and intradimensional set shifting impairments in rats [13,14]. These findings have led to the proposal that THC impairs cognitive flexibility. This is a nebulous term; for the purpose of this paper we define cognitive flexibility as the ability to change response strategies when it is advantageous to do so. Cognitive flexibility is influenced by distinct neural processes that may compete or cooperate to modulate behaviour [15]. For example, animals can learn to select options based on action value estimates acrued over many trials (reinforcement learning), or could instead use heuristics to guide choice [16]. Animals may also rely on innate strategies such as the propensity to switch choices after reward omission, a widespread phenomenon termed lose-shift responding [17–20]. These and other systems can be used to derive choice, and can all manifest as cognitive flexibility.

A common methodological feature in reversal learning and set shifting paradigms is a sudden and unexpected change in reward contingencies, which is used to assess how animals adapt choice strategy. Both lose-shift and reinforcement learning strategies are likely engaged in these paradigms. A physiological mechanism thought to be important for such reinforcement-driven response adaptations is the reward prediction error (RPE) signaling properties of midbrain dopamine neurons [21–23]. These neurons briefly increase firing rate following unexpectedly good reinforcements (rewards), signaling a positive RPE. Conversely, these neurons decrease firing following unexpectedly poor

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reinforcements such as reward omission, signaling a negative RPE. This RPE signal is sufficient to solve a variety of difficult tasks [24]. Drugs or diseases that affect dopamine transmission are therefore expected to impair this prediction error, and thereby impair reinforcement learning, which has been demonstrated in many species and tasks [25–28].

Similar to other drugs of abuse, THC increases the firing rates of midbrain dopamine neurons [29] and increases dopamine release in downstream targets such as the ventral striatum [30]. THC acts as an agonist at the Cannabinoid Receptor 1 (CB1), which is G_i coupled and acts as an inhibitory presynaptic regulator of neurotransmitter release [31]. CB1 is located on inhibitory afferents to dopamine neurons [32]. It is therefore likely that CB1 agonism reduces inhibitory input to dopamine neurons. We expect that this will suppress the pause in dopamine neuron firing following reward omission and thereby attenuate the negative RPE. This should attenuate processes driven by reward omission, such as lose-shift responding and reinforcement learning from worse-than-expected reinforcements. We have previously shown that lose-shift is attenuated by acute administration of amphetamine, which increases striatal dopamine among other effects [26]. We expected that THC would have a similar attenuating effect on lose-shift due to reduction of negative RPE. Furthermore, we expect that THC would impair reinforcement learning from reward omission for the same reason. Learning from positive reinforcements, however, should remain intact [25].

To test this hypothesis, we systemically injected rats with THC and analyzed specific features of reinforcement-driven response adaptation in two reward-based decision-making tasks. The first was a binary choice task that rewards random choice; rats nonetheless employ a win-stay/lose-shift strategy [33]. The second was an uncued reversal task that can be solved using reinforcement learning. These tasks allow us to differentiate between the potential effects of drug on lose-shift responding that occurs on a trial-by-trial basis, and on the multi-trial learning required to track reversals of asymmetric reward probabilities. We found that systemic THC administration attenuated lose-shift responding on both tasks. However, the drug did not impair the ability to flexibly reverse choice preference in response to uncued reversals of reward probability. These results suggest that THC causes an apparent reduction in cognitive flexibility by impairing trial-by-trial loss sensitivity while sparing slower multi-trial learning from wins. This dissociation is supported by recent computational analyses of rodent and human choices in decision-making tasks, which have demonstrated that hybrid models with rapid and gradual components more accurately capture the choices of humans and rodents than do models with a unitary timeframe [34–37].

2. Methods

2.1. Animals

Subjects were 21 adult (90 days old) female Long-Evans rats (bred in-house) weighing 200–250 g. Animals were pair-housed in a climate-controlled vivarium under a 12:12 h light:dark cycle (lights on 7:30 a.m.). Animals had restricted access to water (one hour) on behavioural testing days, but otherwise had ad libitum access to food and water. All procedures were approved by the University of Lethbridge Animal Welfare Committee (AWC) in accordance with the guidelines of the Canadian Council on Animal Care. Our utilization of female rats fills knowledge gaps and is encouraged by the AWC.

2.2. Behaviour apparatus

Behavioural testing was performed in aluminum operant conditioning chambers (see Fig. 1) as described previously [33,36]. Briefly, rats were placed in the operant conditioning chamber for 45 min sessions. Trials were self-paced, and initiated by the rat performing a nose-poke into the central port. Following 150 ms of nose-poke entry, a tone (6 kHz) was presented to indicate that the animal could then locomote to one of the two adjacent sucrose delivery feeders. If the correct feeder was chosen, a reward (60 μL of 10% sucrose solution) was delivered. If the incorrect feeder was chosen, no sucrose was delivered, the house-light illuminated, and the two panel lights extinguished. The state of the lights then reverted (house-light turned off; panel light turned on). This change in lighting served to indicate that reward was not forthcoming, and was of sufficiently short duration such that it terminated by the time the rats returned to the central poke port; there was therefore no 'time-out' associated with reward omission. Once a feeder was chosen, or if no feeder was chosen in the 15 s following a nose-poke, the trial ended and the rat had to return to the central port to initiate a new trial.

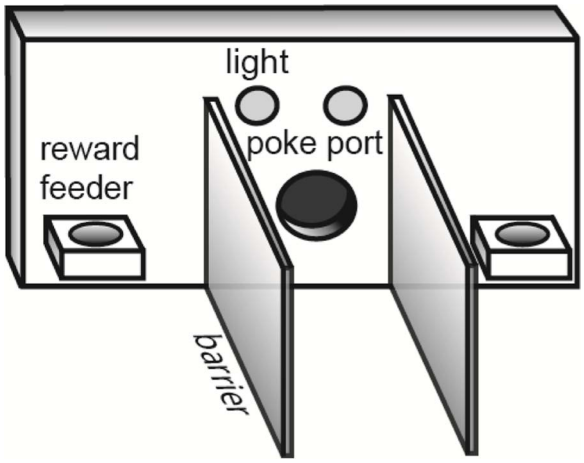

Fig. 1. Schematic diagram of the operant conditioning chamber used for behavioural tasks.

2.3. Experiment 1: acute effects of THC on the Competitive Choice Task (CCT)

The behaviour of animals in the first cohort (n = 10) was shaped during the first two training sessions. All trials were rewarded and no barriers were present in the first training session to facilitate task acquisition. In the second training session, rats were rewarded on 50% of the trials regardless of feeder choice. In all subsequent sessions, reinforcement was controlled by an algorithm that attempted to minimize the number of rewards given to the rats by predicting which feeder the rat would select. This was done by examining the choices and reinforcements from the previous four trials [20,36]. If either feeder was selected at a greater than chance rate in the context of these past trials, it would be unrewarded for the upcoming trial. In doing so, the competitive mode implements the classic 'Matching Pennies' task. Optimal performance (random responding) will result in reward on 50% of the trials. Parallel barriers positioned between the central port and feeder wells were added to introduce a choice cost and discourage feeder bias due to body position. Increasingly longer barriers (4.0, 8.5, 13.5 cm) were introduced during consecutive days of training. Rats were trained until they completed two consecutive sessions of at least 150 trials with the long barriers. All subsequent training and testing sessions were run with the long barriers.

After initial shaping (9 daily sessions), animals were randomly divided into four groups to receive acute THC (Cayman Chemicals, Ann Arbour, MI) in a counterbalanced block design. The drug was dissolved into a 1:1:1:16 solution of THC:ethanol:Cremaphor EL:sterile saline (0.9%) and delivered by intraperitoneal (IP) injection at one of three dosages (0.5 mg/kg, 1.0 mg/kg, or 2.0 mg/kg). Injections were administered 30 min prior to testing on the behavioural task over a period of 8 days using the following schedule: vehicle, injection 1, no injection, injection 2, no injection, injection 3, no injection, and injection 4. The initial vehicle injection served to habituate animals to the

procedure and was not included in the analysis. Injection days consisted of one of the three THC dosages or vehicle. Injecting drug or vehicle every other day provided time for THC to metabolize between sessions in order to reduce the potential confound of extended drug effects [38]. Running the rats on the task with no treatment every other day also provided an opportunity to 'wash-out' any learned choice strategy during the drug sessions.

2.4. Experiment 2: acute effects of THC on the reversal task

An additional cohort of female rats (n = 11) performed a reversal learning task in the same operant conditioning chambers. Animals were trained on the same schedule as cohort 1. Trial initiation was self-paced and began with a nose-poke in the central port. A non-informative tone then prompted the rat to select a sucrose delivery feeder. Correct feeder choices were rewarded with sucrose solution. No reward was given for incorrect choices. In contrast to the Competitive Choice Task, the probability of receiving a reward at a particular feeder was fixed over blocks of 60 trials to either a high or low reward probability. These reward probabilities reversed at the beginning of each block of trials. For example, the left feeder would be the high reward probability side on trials 1–60, and would then reverse to become the low reward probability side for trials 61–120. The reversals repeated several times per session, and the initial location of high-probability feeder was assigned randomly each session. Animals were trained on this task for six sessions, with the high/low reward probability increasing every two sessions from 0.6/0.4 to 0.7/0.3, and finally to 0.8/0.2.

Animals were then randomly divided into two equal groups and received either THC (2.0 mk/kg) or vehicle 30 min before testing in a counterbalanced design with reward probabilities for testing sessions set at 0.8/0.2. The injection schedule across sessions was as follows: vehicle, no injection, injection 1, no injection, injection 2. The initial injection of vehicle was to habituate animals to the process and was not included in the analysis.

2.5. Behaviour analysis

Data were analyzed using Matlab version 2013a (Mathworks, Massachusetts), Graphpad Prism version 6 (Graphpad, La Jolla, CA), and SPSS version 21 (IBM, North Castle, NY). To minimize potential confounds, all trials in which the animal sampled both feeders on the previous trial, which we have previously termed impulsive feeder approach [26], were omitted from the analysis of lose-shift and win-stay probabilities. For Experiment 1 (Acute effects of THC on the Competitive Choice Task), we examined several features of responding including lose-shift, win-stay, reaction time (duration of nose-poke in central port), number of trials, response time (time to locomote from central port to reward feeder), response entropy, intertrial interval, and impulsive feeder approach. Lose-shift responding is a measure of loss sensitivity wherein animals chose to shift feeder choice from that of the previous trial if they failed to receive reward. Win-stay is the re-selection of the feeder on the current trial that provided a reward on the previous trial. Response entropy is a measure of the randomness of an animal's choice sequence [36], with higher values representing more unpredictable responses. This was determined by calculating the Shannon entropy of the animal's choice sequence binned into four consecutive trials [39]. This resulted in nearly random feeder selection having entropy approaching four bits, while any pattern in feeder choice or side bias resulted in a lower value. Note that response entropy is only weakly related to win-stay and lose-shift responses because the competitive algorithm randomizes the rewarded side on a trial-by-trial basis when the rat is performing well. The resultant choice sequence thus appears random even in the presence of strong reward sensitivity. Statistical significance for all measures was determined by one-way repeated measures ANOVAs. The Greenhouse-Geisser correction has been applied to these calculations (Exp 1: ITI, reaction time) when the sphericity assumption was violated, as determined by Mauchly's Test of Sphericity ($\alpha = 0.05$). One animal was removed from Experiment 1 (Acute effects of THC on the Competitive Choice Task) due to being an extreme outlier on most behavioural measures, as determined by Grubb's test ($\alpha = 0.05$).

In Experiment 2 (Acute effects of THC on the Reversal Task), session-wide averages of the same measures were analyzed using paired *t*-tests because only one level of drug was administered. Two-way repeated measures ANOVAs were used to assess the statistical significance of drug and the choice of high/low reward probability feeder. To quantify changes of behaviour within each block of 60 trials irrespective of feeder choice, we collapsed across blocks and binned trials in groups of ten. Each bin was averaged to get a mean value per bin for each animal prior to computing group statistics. Two-way repeated measures ANOVAs were also used to determine statistical significance of drug and bin number (learning) within blocks. All figures display group means and standard error of the mean.

3. Results

3.1. Experiment 1: acute effects of THC on the Competitive Choice Task

Ten female Long-Evans rats were trained to perform a competitive binary choice task (see Methods). We first assessed the potential effects of THC on motivation and motor output. THC had no effect on the intertrial interval ($F(1.7,14.7) = 1.65$, $p = 0.22$; Fig. 2A), or the number of trials performed ($F(3,30) = 1.21$, $p = 0.32$; Fig. 2B). Response time ($F(3,30) = 2.628$, $p = 0.07$; Fig. 2C) and reaction time ($F(1.2,11.9) = 2.78$, $p = 0.12$; Fig. 2D) showed a non-significant tendency to increase as drug dose increased. Finally, the number of pre-reward anticipatory licks at the feeder was also unaffected by THC ($F(3,30) = 0.323$, $p = 0.80$). Together, these results suggest that THC had a minimal effect on motor output and motivation.

The optimal strategy in this task is to generate random choices from trial to trial, and to ignore losses and rewards on previous trials. Overall, THC did not affect the percentage of trials that were rewarded ($F(3,30) = 1.34$, $p = 0.28$; Fig. 3A). However, previous work demonstrated that rats persistently engage the lose-shift strategy on this task [33,36]. Here we found that systemic THC decreased lose-shift responding in a dose-dependent manner ($F(3,30) = 5.34$, $p = 0.005$; Fig. 3B), similar to the effect of systemic AMPH [26]. Additionally, win-stay responding also decreased with increasing THC dose ($F(3,30) = 3.52$, $p = 0.03$; Fig. 3C), while the tendency of animals to impulsively approach feeders without first performing a nose-poke was unaffected ($F(3,30) = 1.57$, $p = 0.22$). THC did not affect the randomness of choice as measured by the response entropy ($F(3,30) = 0.598$, $p = 0.57$; Fig. 3D), suggesting that THC affected trial-by-trial win and loss sensitivity, rather than altering overall response pattern or strategy.

3.2. Experiment 2: acute effects of THC on the Reversal Task

We next sought to determine if THC affected the ability to alter choice strategy in response to changing reward contingencies. Eleven female Long-Evans rats were trained to perform an uncued serial reversal learning tasks in which the reward probabilities (0.8 on one feeder, 0.2 on the other) reversed every 60 trials (see Methods). THC or vehicle was administered 30 min prior to testing over two sessions in a counterbalanced design. The optimal strategy in this task is for animals to develop a strong bias for the feeder with a higher probability of reward. They must also have the flexibility to switch this bias when a new block of trials begins and feeder reward probabilities reverse. Consequently, they could earn reward on nearly 80% of the trials by choosing optimally, but would earn reward on only 50% of trials by choosing randomly. In practice, animals acquired rewards on 66 ± 3% of trials (see Fig. 4), indicating that they did alter their choice biases in

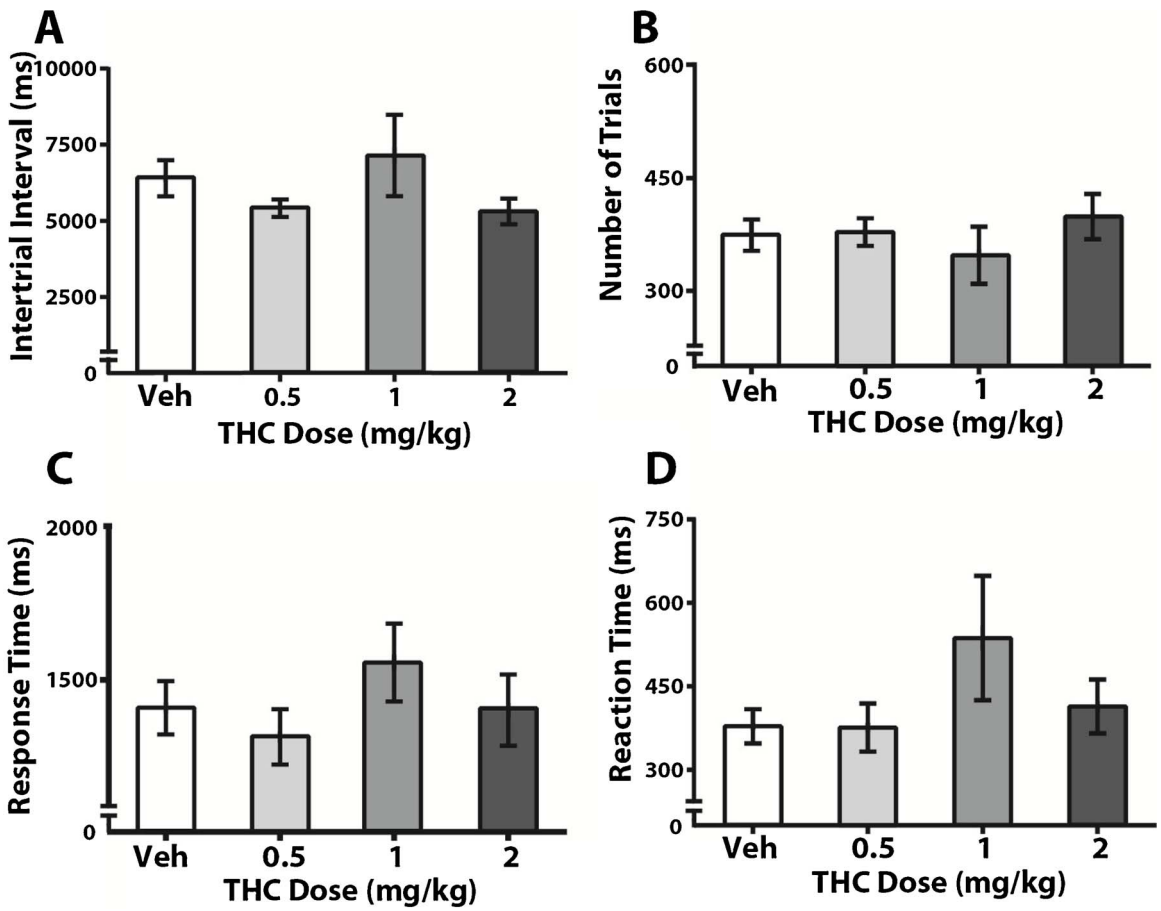

Fig. 2. Motoric and motivational effects of THC on the Competitive Choice Task. (A) THC did not affect the time between trials, as indicated by intertrial interval. (B) Group-averaged number of trials, which was unaffected by drug. (C) The time to locomote from the central port to the reward feeder was unaffected by THC. (D) The length of time spent in the central nose port in order to initiate a trial was unaffected by THC. Error bars indicate SEM.

order to exploit asymmetries in reward probability.

Similar to Experiment 1, drug administration did not significantly affect the number of trials per session ($t(10) = 1.65$, $p = 0.13$; Fig. 5A), intertrial interval ($t(10) = 1.23$, $p = 0.25$; Fig. 5B), or pre-reward anticipatory licks ($t(10) = 0.07$, $p = 0.95$), further suggesting no gross impairment in motor control or motivation. However, reaction time ($t(10) = 2.50$, $p = 0.03$) and response time ($t(10) = 2.43$, $p = 0.04$) increased with THC administration. Similar to Experiment 1, systemic THC decreased lose-shift responding ($t(10) = 3.47$, $p = 0.007$; Fig. 5C). In contrast to Experiment 1, win-stay responding *increased* following THC injection with increasing drug dose ($t(10) = 2.37$, $p < 0.05$; Fig. 5D), while impulsive feeder approach was again unaffected by drug ($t(10) = 0.059$, $p = 0.95$). Futhermore, THC administration reduced response entropy ($t(10) = 3.83$, $p = 0.004$; Fig. 5E). This can likely be explained by the fact that THC increased the probability of the rat returning to the previous feeder choice (high reward probability) regardless of choice outcome ($t(10) = 3.38$, $p = 0.008$; Fig. 5F). This is a good strategy in this task and thus not considered an impairment.

Next we sought to determine if responding was affected by whether the animals had selected the high or low reward probability feeder on either the previous or current trial. Group means for lose-shift and win-stay responding were computed based on whether the animals had selected the high or low probability feeder on the previous trial. Group means were also computed for response time and pre-reward anticipatory feeder licks based on the high/low reward probability feeder selection on the current trial. Two-way repeated-measures ANOVAs were used to analyze the effect of drug and feeder choice. Rats generated much more anticipatory licking when they selected the high reward probability feeder (main effect of feeder choice: $F(1,9) = 65.8$, $p < 0.0001$; Fig. 6A), but THC did not affect anticipatory licking (main effect of drug: $F(1,9) = 0.085$, $p = 0.78$; Fig. 6A). Furthermore, response times were lower (running velocity was higher) when approaching the high reward probability feeder (main effect of feeder choice: $F(1,9) = 5.49$, $p = 0.04$; Fig. 6B). THC appeared to have no effect on response times (main effect of drug: $F(1,9) = 3.46$, $p = 0.10$; Fig. 6B). Together, these data indicate that THC had no significant effect on motivation or the ability of rats to discriminate which of the feeders was more likely to deliver reward. Feeder choice had a significant effect on lose-shift responding (main effect of feeder choice: $F(1,9) = 63.8$, $p < 0.0001$; Fig. 6C), such that animals were much more likely to lose-shift after receiving a loss at the low reward probability feeder than the high probability feeder. Consistent with session-averaged data (Fig. 4C), lose-shift responding decreased with THC administration regardless of the reward probability (main effect of drug: $F(1,9) = 10.4$, $p = 0.01$; Fig. 6C). The prevalence of win-stay responding was also higher following wins at the high reward probability feeder (main effect of feeder choice: $F(1,9) = 10.8$, $p = 0.009$; Fig. 6D), and this was unaffected by drug (main effect of drug: $F(1,9) = 1.94$, $p = 0.20$; Fig. 6D). These data suggest that feeder choice has a much greater effect on our task measures than did THC.

We next sought to determine whether the measures of choice varied across trials within a block, independent of feeder choice. We collapsed data across blocks, and aggregated trials into six bins of 10 trials. Two-way repeated-measures ANOVAs were used to analyze the effect of drug and bin number. Our data suggests that rats adapted to the changing reward probabilities. They were more likely to receive a reward in the later trial bins, as compared to early trial bins, within each block (main

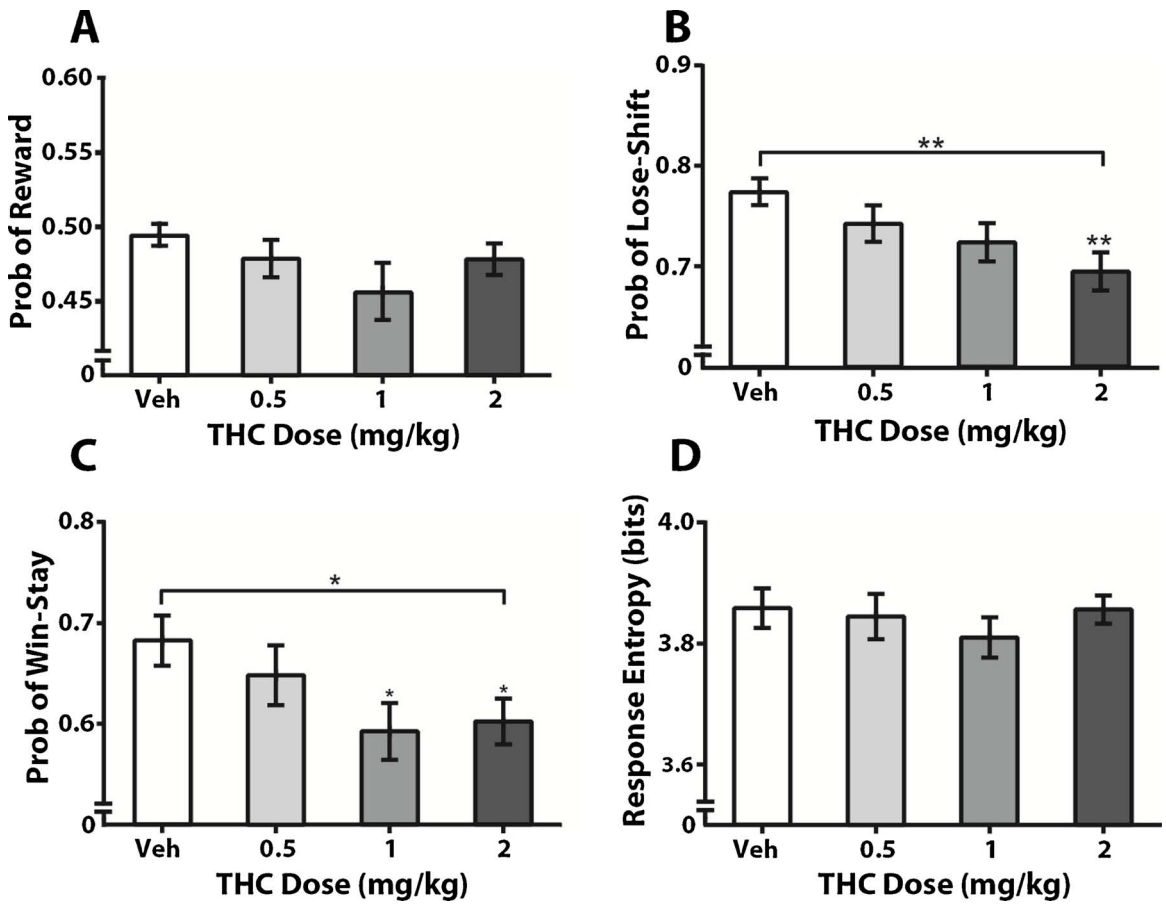

Fig. 3. Session-averaged effects of acute THC administration on performance measures in the Competitive Choice Task. (A) THC did not affect the percentage of trials that were rewarded. (B) Probability of lose-shift responding decreased in a dose-dependent manner with THC administration. (C) Probability of win-stay responding, which was also reduced by systemic THC. (D) THC had no effect on the randomness of feeder selection over trials, as assayed by response entropy. Statistical significance for RM-ANOVAs are indicated by '*' for $p < 0.05$, and '**' for $p < 0.01$. Asterisks above individual points indicates group means that were significantly different from the control (Vehicle) mean according to Dunnett's post hoc test with $\alpha = 0.05$. Error bars indicate SEM.

effect of bin number: $F(5,45) = 21.5$, $p < 0.0001$; Fig. 7A). Drug had no effect on the probability of receiving a reward (main effect of drug: $F(1,9) = 0.003$, $p = 0.95$; Fig. 7A). Animals were also less likely to switch their feeder choice from the previous trial, regardless of outcome, as trials progressed within a block (main effect of bin number: $F(5,45) = 3.16$, $p = 0.02$; Fig. 7B), and this was further decreased by drug (main effect of drug: $F(1,9) = 12.6$, $p = 0.006$; Fig. 7B). Consistent with the session-averaged analysis, drug had a significant effect

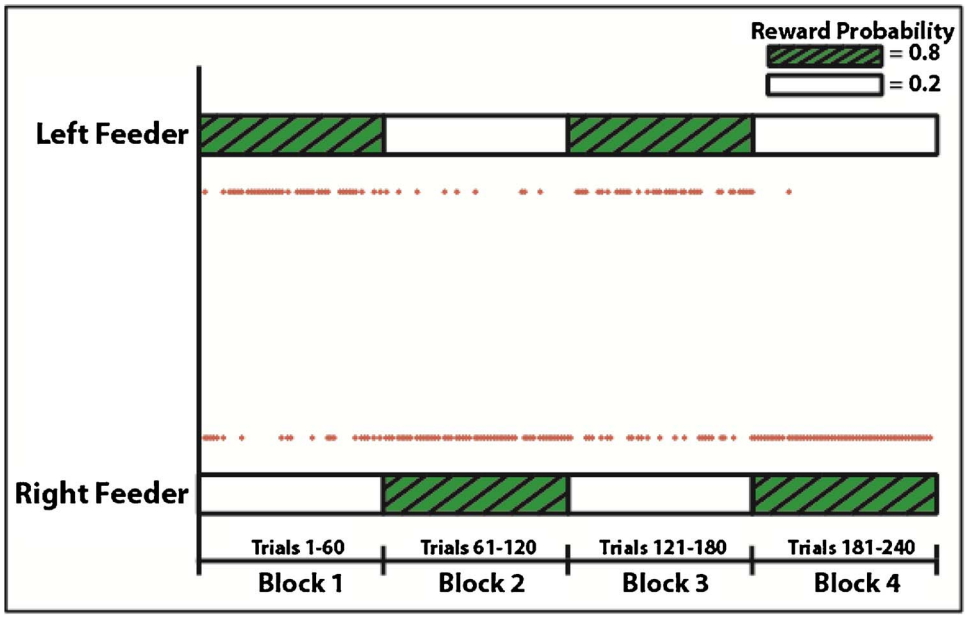

Fig. 4. Feeder selection for one representative animal during a session of the Reversal Task (240 trials over 4 blocks). Each orange dot represents the feeder selected on a single trial. The choice pattern demonstrates shifting feeder preference in response to changing reward contingencies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

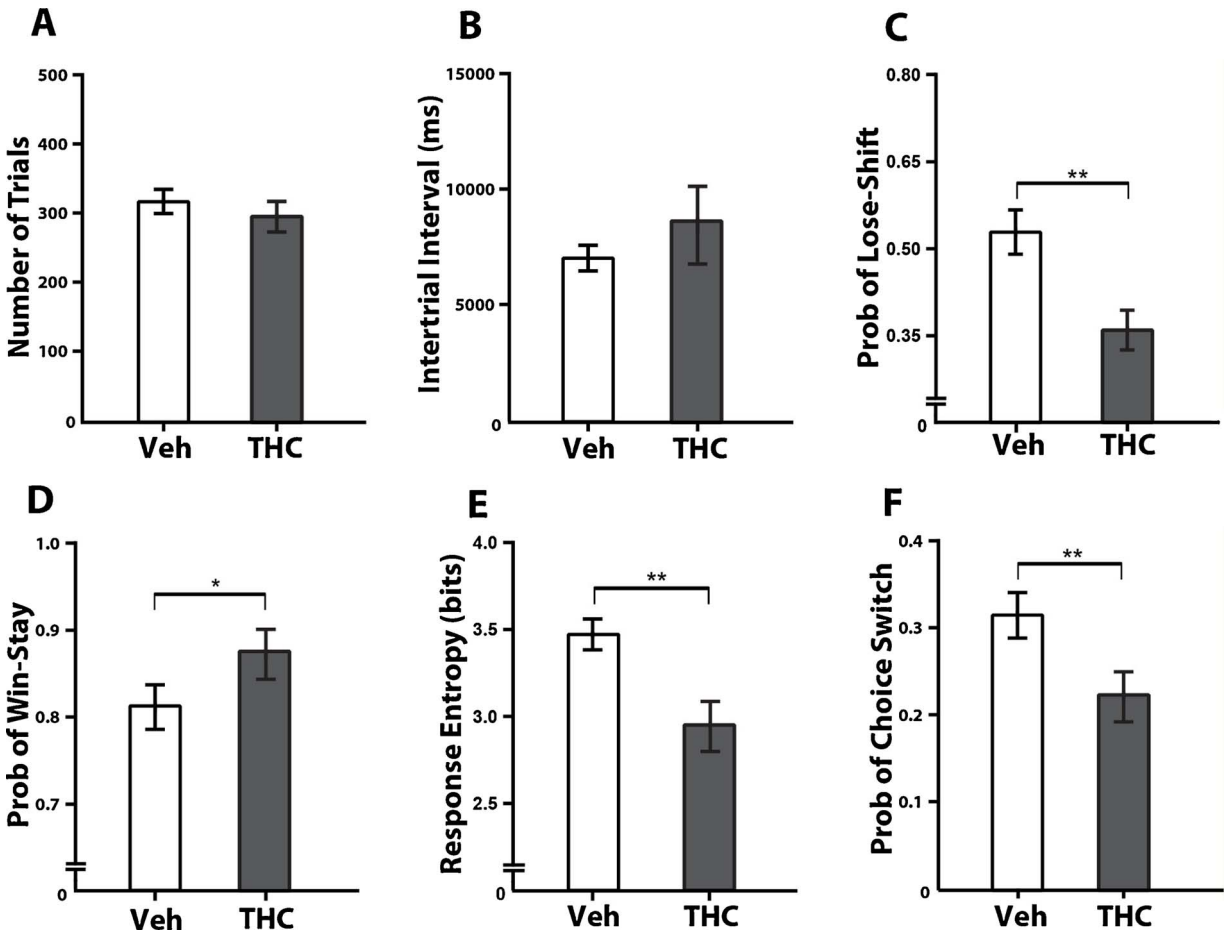

Fig. 5. Treatment-averaged effects of acute THC on behaviour in the Reversal Task. (A) THC had no effect the number of trials performed. (B) THC did not affect the intertrial interval. (C) Probability of lose-shift responding was decreased by systemic THC. (D) Probability of win-stay responding was increased by THC. (E) THC significantly reduced the randomness of feeder selection over trials, as measured by response entropy. (F) The probability of a switch from the previous feeder choice independent of reinforcement was significantly reduced by THC. Statistical significance is indicated by '*' for $p < 0.05$, and '**' for $p < 0.01$ for paired *t*-tests. Error bars indicate SEM.

to reduce lose-shift responding ($F(1,9) = 17.6$, $p = 0.002$; Fig. 7C), whereas bin number had no effect ($F(5,45) = 2.31$, $p = 0.06$; Fig. 7C). Drug also had a significant effect on win-stay ($F(1,9) = 5.28$, $p < 0.05$; Fig. 7D), whereas bin number had a strong effect on win-stay such that it increased as blocks progressed ($F(5,45) = 3.82$, $p = 0.006$; Fig. 7D). These data reveal that while THC did affect short-term decision-making (lose-shift, choice switch, and win-stay), it did not affect the ability to learn to bias choices to high-probability feeders.

4. Discussion

Acute THC has been proposed to impair cognitive flexibility in rats and other animal models based on evidence that it reduces performance on some tests of set shifting and reversal learning [12,14]. Performance on such tasks, however, likely depends on several information processing systems in the brain, such as those involved in short-term memory, reinforcement learning, and impulse control [40]. Here, we sought to refine our understanding of THC's effects on decision-making by analyzing its effects on some of these component systems. In particular, we focused on lose-shift responding to assess immediate short-lasting effects, and reversal learning to assess more gradual learning over many trials. We previously found that lose-shift responding depends specifically on sensorimotor regions of the striatum [36], and is attenuated in a dose-dependent manner by AMPH administration [26]. This effect is likely due to the ability of amphetamine to increase dopamine release in the striatum [41], which is expected to attenuate the brief suppression of dopamine transmission that normally indicates that the reinforcement obtained was less than expected [21]. Because THC also increases tonic dopamine levels, albeit through a different mechanism, we hypothesized that lose-shift would be likewise suppressed by THC. We indeed found such an effect (Figs. 3 and 5). Lose-shift responding on this task depends on a memory trace that decays over 7 s [33]. Therefore, the present data suggest that THC will impair behavioural control that benefits from rapid, but short-lasting, choice shifting away from poor response options.

Given its rapid decay, lose-shift responding appears to be distinct from other forms of reinforcement learning that contribute to decision-making based on reinforcement delivery over many trials and much longer timescales [33]. We tested if acute THC affected this gradual learning by evaluating the ability of rats to flexibly reverse response biases so as to track uncued reversals of reward probability. We found that THC had no effect on the slower form of reinforcement learning needed to solve the Reversal Task (Fig. 7). This is illustrated by the fact that THC did not reduce the overall reward obtained during sessions or impair the ability to follow uncued reversals of reward probability.

Our findings are in contrast to other studies reporting an effect of THC on reversal learning and set shifting [12,13]. It is unlikely these discrepancies are due to drug dose effects, as these studies found impairments in set shifting [12] in lower doses (0.1–0.5 mg/kg), and reversal learning deficits at equivalent doses (2.0 mg/kg) [13] compared to those used in our study. We note that these other studies used discriminative cues, which were omitted in the present study. It is possible that the addition of these task features recruits additional brain regions that are affected by THC. One such region is the orbitofrontal cortex,

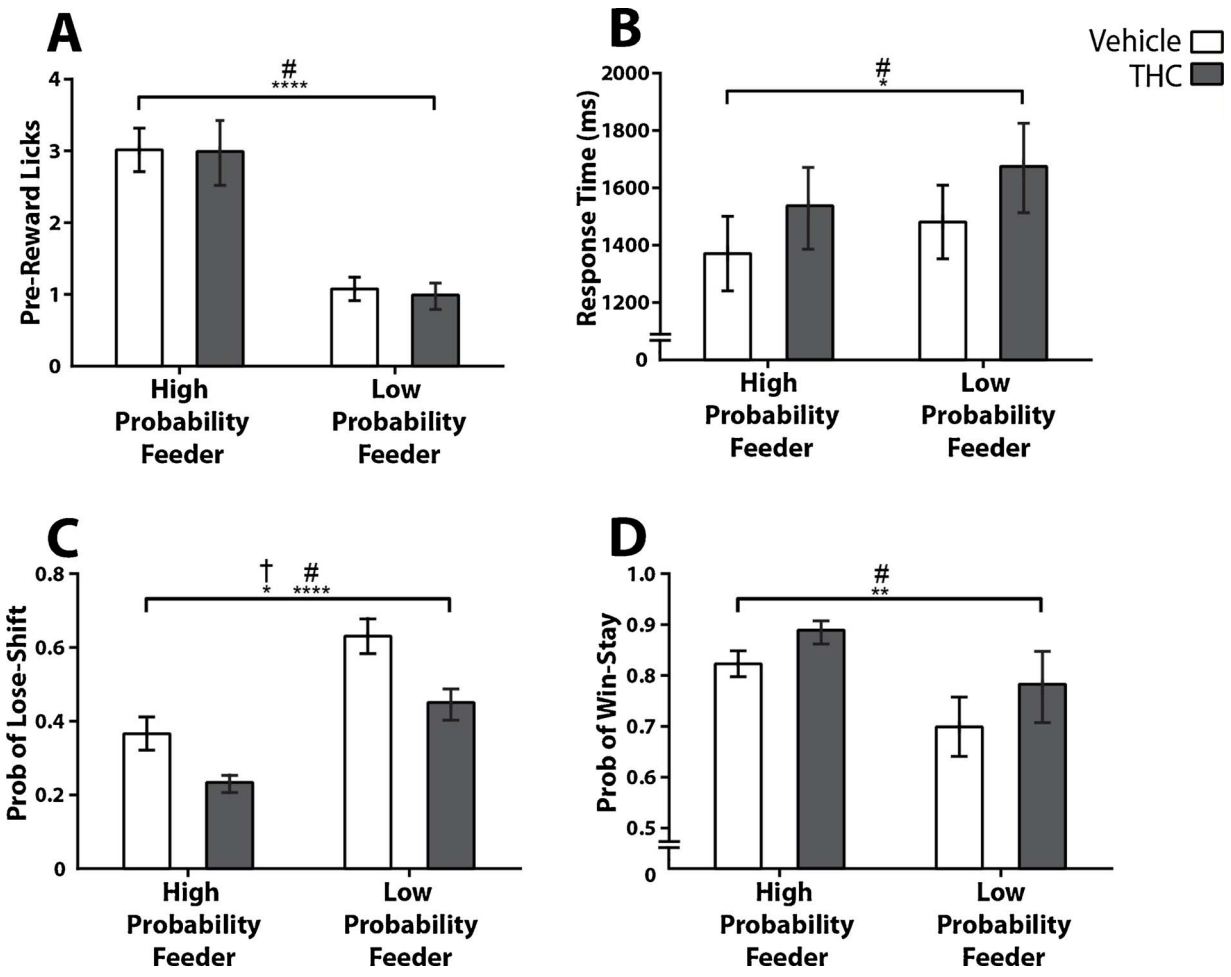

Fig. 6. Effects of acute THC and feeder choice on behavioural measures in the Reversal Task. (A) Anticipatory licking was increased when the high reward probability feeder was chosen. (B) Response time was reduced on trials where the high reward probability feeder was chosen. (C) THC reduced lose-shift, which was higher at the low probability feeder. (D) Win-stay responding was lower at the low reward probability feeder. Significance was determined by a two-way RM-ANOVA indicated by '*' for $p < 0.05$, '**' for $p < 0.01$, '***' for $p < 0.001$, and '****' for $p < 0.0001$. A main effect of feeder choice is indicated by '#', while a main effect of drug is indicated by '†'. Error bars indicate SEM.

which is involved in updating stimulus-outcome associations [42], and has increased immediate early gene activity following THC administration [13]. This leads us to speculate that animals are less reliant on the orbitofrontal cortex to perform our uncued tasks, thus leading to possible THC-induced changes in the region having little effect in task performance. Furthermore, rats do sometimes receive reward on the low-probability feeder in the present task. This differs from most reversal learning tasks, which tend to provide no reward (or aversive reinforcement such as quinine) on the previously optimal choice after a reversal has occurred. Another possibility is that sex differences influenced our results, although we argue this is unlikely. We have controlled for sex in Long Evans rats performing the Competitive Choice Task and found only minor differences unrelated to the main findings here (unpublished observations). Moreover, we expect little to no effect of estrous cycle because an extensive meta-analysis of behavioural and physiological traits found equal variability in both sexes, even when including estrous cycle phases [43]. We thus expect that the effects of THC reported here would replicate in male rats. Our finding that THC does not affect uncued reversal learning suggests that THC only affects certain subsystems involved in cognitive flexibility.

Rats in the present study discriminated the expected value of the feeders in the Reversal Task; they exhibited increased win-stay responding, increased pre-reward licks, and more quickly approached the high probability feeder as compared to the low probability feeder. Rats also exhibited less lose-shift responding at the high probability feeder. This reduction is a favorable response policy and suggests that some processing systems involved in behavioural flexibility can inhibit others. Because lose-shift responding depends on the dorsolateral striatum [36], we speculate that the advantageous reduction of lose-shift involves the suppression of this system by the prefrontal cortex. Such suppression of innate behaviours by executive systems has been suggested by others [44,45].

While THC and AMPH both impair lose-shift responding, their effects on behaviour differed in several respects. For example, AMPH decreased reaction time [26], whereas THC increased reaction time and response time in the Reversal Task, and showed a non-significant trend to increase these measures in the Competitive Choice Task (Table 1). Furthermore, AMPH had no effect on win-stay responding, while THC decreased this behaviour in the Competitive Choice Task and increased it in the Reversal Task. Given the weak and opposing effects on win-stay, we cannot rule out the possibility of a spurious effect due to the binary nature of many deductive statistical tests [46]. It is also possible that win expectancy of the rats differs between the tasks.

In contrast to win-stay responding, the tendency to approach feeders outside of the trial period to seek rewards was unaffected by THC. Animals should always return to the central nose-poke after responding at a feeder. However, on some trials they would instead approach the alternate feeder presumably seeking a reward. This was never reinforced, and so we argue that it is an impulsive behaviour [26] that limits total reward during a session. This impulsive feeder approach is dramatically increased by systemic amphetamine, or by infusions of amphetamine into the ventral striatum [26], or by lesions of the

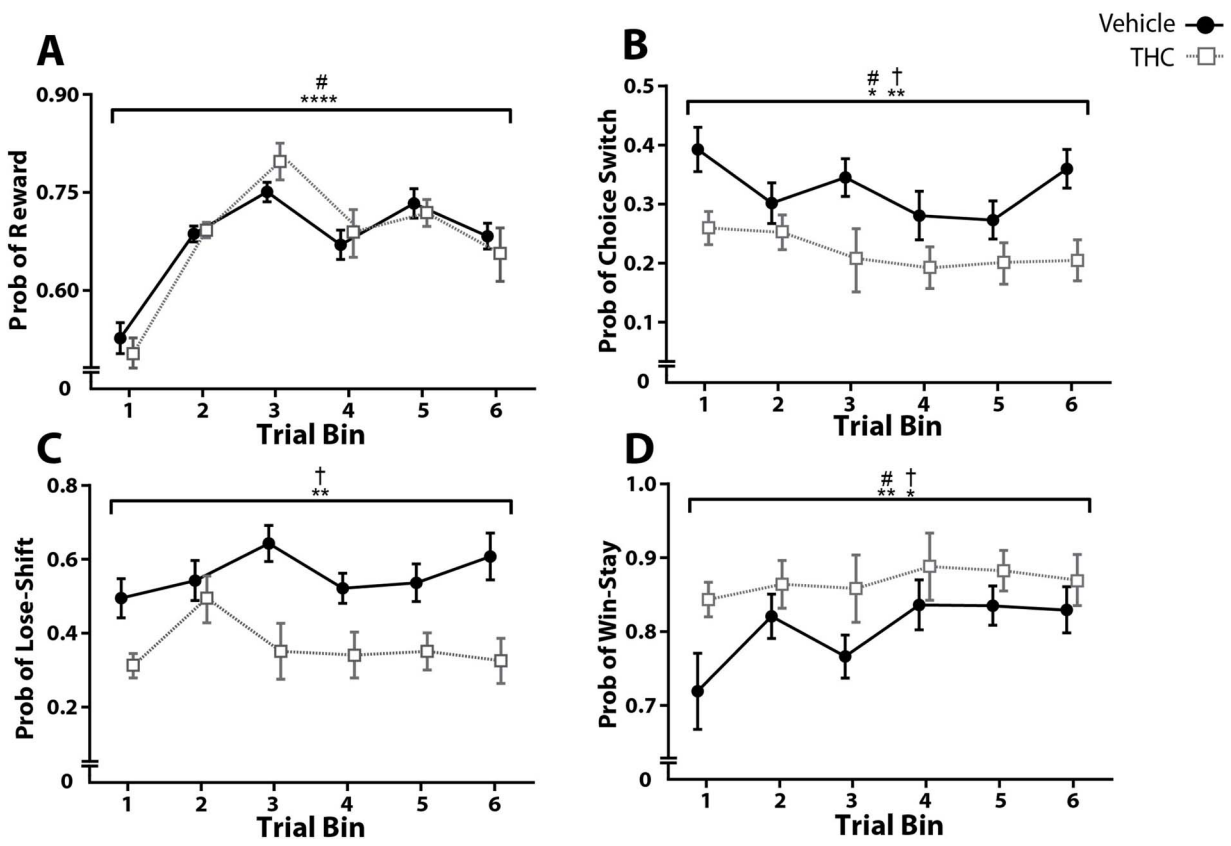

Fig. 7. Within-block analysis of THC's effects on behavioural measures in the Reversal Task. (A) The probability of reward was strongly affected by bin number. (B) The probability of a switch from the previous choice, independent of reinforcement, was affected by both drug and bin number. (C) THC reduced lose-shift responding, while bin number had no effect. (D) Drug and bin number both had effects on win-stay responding. Significance as determined by a two-way RM-ANOVA is indicated by '*' for $p < 0.05$, '**' for $p < 0.01$, and '****' for $p < 0.0001$. A main effect of drug is indicated by '†', while a significant main effect of bin number is indicated by '#'. Error bars indicate SEM.

Table 1
Comparison of behavioural measures influenced by THC in the Competitive Choice and Reversal Tasks. Strong effects are denoted with two arrows, while moderate effects are denoted with one arrow. Arrow directions indicate directionality of differences as compared to vehicle injections. Non-significant effects are denoted with '-'.

Behavioral Measure	Competitive Choice Task	Reversal Task
Lose-Shift	↓↓	↓↓
Win-Stay	weak ↓	weak ↑
Impulsive Feeder Approach	–	–
Response Entropy	–	↓↓
Number of Trials	–	–
InterTrial Interval	–	–
Reaction Time	–	↑
Response Time	–	↑
Reversal Tracking	N/A	–

sensorimotor striatum (unpublished data). We therefore speculate that it is a form of goal tracking in Pavlovian approach (which increases with systemic amphetamine [47]) that may normally be suppressed by the sensorimotor striatum. The expression pattern of CB1 mRNA provides a possible explanation for why THC reduces lose-shift responding, but has little effect on impulsive feeder approach. CB1 mRNA expression has a ventromedial to dorsolateral gradient in the striatum, with greater expression towards the dorsal regions of the lateral striatum [48–50]. While CB1 mRNA is expressed primarily in the dorsolateral striatum (DLS), immunohistochemistry experiments have demonstrated that the CB1 receptor itself is primarily located on the corresponding medium spiny neuron afferents located in the substantia nigra [32]. This leaves DLS CB1 receptors ideally placed to presynaptically modulate medium spiny neuron-driven inhibition of substantia nigra dopamine release and influence dopaminergic prediction errors. Conversely, relatively little CB1 mRNA is expressed in the ventral striatum this may be an explanation for why ventral striatum-driven impulsive feeder approach was unaffected by THC. Further experiments will be needed to properly test this hypothesis.

As with all systemic pharmacology experiments, we cannot ascertain which brain structures are affected and can only speculate on potential neurobiological mechanisms. While CB1 expression is greatest in the striatonigral pathway, amygdala, and hippocampus, moderate expression also occurs in the neocortex [31]. Nonetheless, our results are consistent with human studies concluding that THC does not cause global cognitive impairments [51,52]. Rather, our results suggest that acute THC administration instead primarily affects specific components of the brain's decision-making systems.

5. Conclusions

The data presented here reveal that THC reduces the immediate responses to losses, but not the ability to track uncued reversals of reward probability. This suggests that THC is capable of affecting a subset of dissociated processes that contribute to behavioural flexibility. We have shown that this occurs both in a competitive choice task, where insensitivity to losses and wins is the optimal strategy, and in a reward probability reversal learning paradigm, where avoiding losses and chasing wins is the optimal strategy. Thus, at least in some tasks, THC does not impair overall cognitive flexibility, but does selectively reduce avoidance of choices associated with recent losses.

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