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Endocannabinoid Involvement in Impulsivity and Decision-making

Christopher P. Norris
Wilfrid Laurier University, cprnorris@gmail.com

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by

Christopher P. R. Norris

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Abstract

Problem gambling is a widespread phenomenon with a prevalence estimate of 2.3% globally (Williams, Volberg, & Stevens, 2012). Although little is known about the neurochemistry underlying this pathological behaviour, evidence suggests that dysregulation of the brain's endocannabinoid (eCB) system may be implicated in impulsivity and decision-making. For example, chronic cannabis users exhibit impulsive behaviour and impaired decision-making on the Iowa Gambling Task (IGT). The present study sought to further examine the role of the eCB system in problem gambling-related decision-making in laboratory rats using the five-choice serial reaction time task (5-CSRTT), and a recently-developed rodent analogue of the IGT called the rat gambling task (rGT). It was predicted that increasing neural levels of the eCB anandamide by administering the fatty acid amide hydrolase (FAAH) inhibitor URB597 would increase impulsivity as found previously with psychomotor stimulants. Results revealed that URB597 (0.03-1 mg/kg, IP) had no effect on premature responding or correct choices. Cocaine (15 mg/kg, IP) increased premature responding and decreased choice accuracy in the 5-CSRTT, but these effects were not attenuated by the CB1 inverse agonist rimonabant (3 mg/kg, IP). Furthermore, neither URB597 (0.03-1 mg/kg, IP) nor the cannabinoid receptor agonist THC (1.0-1.5 mg/kg, IP) altered optimal choice preference or premature responding in the rGT. Taken together, results did not support the notion that eCBs are involved in impulsivity or decision-making. We also conclude that any involvement of the eCB system in impulsivity is likely a downstream process from dopamine release.

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List of Abbreviations:

5-CSRTT: five-choice serial reaction time task

5-HT: Serotonin

ADHD: Attention Deficit/Hyperactivity Disorder

CB1R: cannabinoid receptor subtype 1

CB2R: cannabinoid receptor subtype 2

CRF: Corticotrophin releasing factor

DSM: Diagnostic and Statistical Manual of Mental Disorders

FAAH: Fatty Acid Amine Hydrolase

ICD: Impulse Control Disorder

IP: intraperitoneal

ITI: Inter-trial interval

NMDA: N-methyl-D-aspartate

PEG 400: polyethylene glycol

PG: Problem Gambling

rGT: rat gambling task

SEM: standard error of the mean

TWEEN 80: Polyoxyethylene (20) sorbitan monooleate

THC: Δ^9-tetrahydrocannabinol

URB597: [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate

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Introduction

Many individuals gamble without developing dangerous habits, but problem gambling (PG) remains a significant problem for a substantial number of individuals. The DSM-IV defined PG as an impulse control disorder that presents with at least five of the following symptoms: preoccupation, tolerance, withdrawal, escape, chasing, lying, loss of control, illegal acts, risked significant relationship, and bailout. The recently published DSM-5, however, instituted a controversial change and reclassified PG as an addiction on the basis that PG shares many of the same symptoms and problems as substance abuse, and because most problem gamblers, unlike sufferers of Impulse Control Disorders (ICDs), do not report an overwhelming urge to indulge in the behaviour that is relieved by acting out the desire. Most gamblers enjoy the experience while it is occurring, and only afterwards do they feel distress. PG does, however, have high comorbidity with impulse control disorders such as kleptomania and pyromania (American Psychiatric Association, 2013). The drive to begin gambling may be an addictive behaviour, but impulsivity appears to play a role during the act of gambling. Problem gamblers demonstrate impaired ability to inhibit motor responses, a classic measure of impulsivity, but interestingly do not perform worse on another classic measure of impulsivity, the Stroop Task (Brevers et al., 2012; Dannon, Shoenfeld, Rosenberg, Kertzman, & Kotler, 2010)

The controversy in the literature has driven many researchers to examine how impulsivity plays a role in gambling behaviour. The most common test of

impulsivity used in gambling research is the Iowa Gambling Task (IGT). In the IGT participants pick cards from four decks with varying ratios of cards that increase their winnings, and cards that decrease their winnings. Typically, participants discover the optimal deck within 40-50 trials. Many recent studies have focused on identifying factors that influence decision-making in the IGT including personality variables, environmental manipulations, pathological states, and pharmacological manipulations. Problem gamblers, for example, perform substantially worse in the IGT compared to non-gambling controls (Goudriaan, Oosterlaan, de Beurs, & van den Brink, 2005). Additionally, patients with injuries to the prefrontal cortex (Bechara, Damasio, Damasio, & Anderson, 1994), and patients with schizophrenia treated with atypical antipsychotics (Wasserman, Barry, Bradford, Delva, & Beninger, 2012), show deficits in the IGT that are likely related to altered medial prefrontocortical function.

One obstacle to the rigorous study of factors influencing impulsivity is the large variation present in the human population, which is both difficult to categorize and control, as well as ethical considerations that preclude enquiries into neurophysiological and neuropharmacological determinants. Animal models therefore play an increasingly important role in the study of PG-related behaviour. The prototypical task for studying decision-making in rodents is the 5-choice serial reaction time task (5-CSRTT) which was developed by Carli, Robbins, Evenden, and Everitt (1983) to directly model a human 5-choice task used to investigate attention deficit/hyperactivity disorder (ADHD). In the 5-CSRTT, after an initial free reward to facilitate a nose poke into the food cup, an inter trial interval is activated before a

brief flash of light is randomly presented in one of five separate nose-poke apertures. Nose poking into the correct aperture within a certain time limit results in a food reward, while nose poking into an incorrect aperture results in a timeout. A new trial can then be initiated by a nose poke into the food cup. The task requires substantial focus as inattention to any part of the task results in a loss of food reward for that trial. Specifically, inattention to the stimulus location would result in an incorrect response, and inattention to the specific timing of the stimulus results in impulsive premature responding. The 5-CSRTT remains the most common measure of impulsivity and attention in the literature, and is sensitive to pharmacological manipulation. Most psychomotor stimulants have been found to increase premature responding, presumably as a result of increased impulsivity (van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006). One exception, however, is methylphenidate, which increases accuracy. This finding is consistent with the use of this drug as a treatment for ADHD. The same study found that dizocilpine, an NMDA receptor antagonist, impaired accuracy, increased premature responses, and increased omissions. The norepinephrine reuptake inhibitor desipramine decreased premature responses and increased latencies and omissions (Paine, Tomasiewicz, Zhang, & Carlezon, 2007). Additionally, attention disruption by corticotrophin releasing factor (CRF) can be attenuated by a κ-opioid antagonist (Van't Veer, Yano, Carroll, Cohen, & Carlezon, 2012).

Serotonin (5-HT) also appears to play a role in the cognitive processes required for the 5-CSRTT. Complete 5-HT depletion by 5,7-dihydroxytryptamine increased premature responses, and the 5-HT_{1A} agonist 8-OH-DPAT decreased

accuracy (Carli & Samanin, 2000; Harrison, Everitt, & Robbins, 1997). Of particular relevance to the present study is a small but growing literature suggesting that the endocannabinoid (eCB) system also appears to play a role in impulsivity, although the relationship is more complex. Cannabinoid receptor agonists have no effect, but cannabinoid receptor antagonist can reduce baseline impulsivity, and impulsivity induced by other drugs (Pattij et al., 2007; Wiskerke, Stoop, Schetters, Schoffelmeer, & Pattij, 2011; Wiskerke, van Mourik, Schetters, Schoffelmeer, & Pattij, 2012). Taken together, these pharmacological studies demonstrate that impulsivity is a complex process that involves the interaction of many different systems (see reviews in Dalley & Roiser, 2012; Robbins, 2002).

The 5-CSRTT is useful for examining impulsivity in rats, but it is not a direct model of human gambling behaviour. Only recently have researchers attempted to directly model human gambling behaviour, with varying degrees of success. Van den Bos, Lasthuis, den Heijer, van der Harst, and Spruijt (2006) created a radial arm maze that modelled the IGT by rewarding rats with sweetened rice or punishing them with quinine in different ratios in four different arms. Zeeb, Robbins, and Winstanley (2009) simplified the model by designing an operant version and manipulating the size of reward and simply using variable timeout lengths for punishment. They named their task the rat gambling task (rGT) after the IGT.

The optimal strategy in the rGT mirrors that of the IGT; thus, animals are presented with four apertures associated with differential probabilities of reward and punishment, in a manner analogous to the four decks used in the IGT. Performing a nose poke response into the aperture with the most favourable

probabilities (i.e., the optimal choice), yields an 80% chance of a two-pellet reward and a 20% chance of causing a 10-s timeout (P1). The next best option is associated with a 90% chance of a one-pellet reward and a 10% chance of a 5-s timeout (P2). The third option is associated with a 50% chance of delivering three pellets and a 50% chance of causing a 30-s timeout (P3). The final option has a 40% chance of delivering four pellets and a 60% chance of causing a 40-s timeout (P4). Thus, as the probability of reward decreases, the size of the reward and the duration of the timeout period increase.

This task is sensitive to the effect of rearing environment and certain drugs; for example, pair-housed rats are quicker to learn the optimal strategy relative to their isolated conspecifics, and amphetamine impairs the decision making of pair-housed, but not isolated animals (Zeeb, Wong, & Winstanley, 2013). The effects of amphetamine in the rGT mirror its effects in the 5-CSRTT. That is, the drug increases premature responding in both tasks and impairs the ability of rats to perform optimally.

A growing body of evidence now supports the involvement of dopamine in impulsive behaviour (Murillo-Rodríguez, Palomero-Rivero, Millán-Aldaco, Arias-Carrión, & Drucker-Colín, 2011; Pattij & Vanderschuren, 2008; Zeeb et al., 2009, 2013). Drugs that are known to increase dopamine levels consistently increase premature responding in the 5-CSRTT, but differ on many other attentional parameters. Cocaine, amphetamine and nicotine all increase premature responding, but cocaine also increases the number of incorrect choices, suggesting that the drugs do have some unique effects. Additionally, the dopamine D1-like receptor antagonist

SCH 22390 and the D2-like receptor antagonist eticlopride both attenuated drug-induced premature responding, but SCH 22390 only reduced drug-induced premature responding at a dose that reduced baseline premature responding (van Gaalen et al., 2006). Although the dopaminergic system plays a primary role in impulsivity, D1-like and D2-like receptors appear to have distinct roles in the process.

Several studies also support the notion that impulsive behaviour is mediated not only by the dopamine system, but also by other neurotransmitter systems including serotonin, noradrenaline, and glutamate (reviewed by Pattij & Vanderschuren, 2008). Additionally, a few studies have implicated the eCB system in impulsivity. For example, both marijuana and its principle psychoactive ingredient delta-9-tetrahydrocannabinol (THC) increases the incidence of risk-taking behaviour in a laboratory setting (Lane, Cherek, Tcheremissine, Lieving, & Pietras, 2005), and induce impulsive action in a stop signal task (reviewed by Pattij & Vanderschuren, 2008). Moreover, abstinent chronic cannabis users perform worse on the IGT relative to non-using controls, and cannabis use disorder symptoms are associated with poorer decision-making (Gonzalez et al., 2012; Hermann et al., 2009).

Basic understanding of how the eCB system operates at the neural level has advanced rapidly since the identification in the 1990s of the first two cannabinoid receptors (termed CB1R and CB2R). CB1Rs are the most abundant G-protein coupled receptors found in the brain, and given their widespread distribution it is not surprising that the eCB system modulates diverse physiological and behavioural

functions including feeding homeostasis, nociception, motor control, learning and memory. The subjective effects of THC administration are the result of the stimulation of these receptors, although stimulation of the CB1R subtype appears to be primarily related to the subjective effects of cannabis use (Devane et al., 1992; Devane, Dysarz, Johnson, Melvin, & Howlett, 1988; Munro, Thomas, & Abu-Shaar, 1993). Endogenous cannabinoids act as retrograde signalling messengers primarily at glutamatergic and GABAergic synapses, and via their action on G-protein-coupled neuronal CB1Rs regulate voltage-gated Ca++ channels, K+ channels, adenylyl cyclase activity, as well as mitogen-activated kinases. The specific action on these processes is context specific.

Exogenous and endogenous cannabinoids both act on CBRs, but they have unique properties. THC remains in the brain much longer than anandamide, which is quickly broken down by the fatty acid amine hydrolase (FAAH) enzyme (Deutsch & Chin, 1993). This initially made anandamide difficult to study, but Fegley et al. (2005) developed a novel FAAH inhibitor, called URB597, that was able to increase anandamide levels throughout the brain. Researchers have used URB597 by itself and in tandem with anandamide administration to stimulate CBRs in a similar manner to THC. There is also substantial evidence that there is strong interaction between eCB and dopamine systems.

Exogenous cannabinoid administration greatly affects dopamine levels in the brain, but the interaction between the two systems has only been studied in the context of reward and not impulsivity (French, 1997). Anandamide causes a CB1R-dependent spike in dopamine levels when administered intraperitoneally (Murillo-

Rodríguez et al., 2011). Microinfusions of the FAAH inhibitor URB597 into the lateral hypothalamus and dorsal raphe nucleus also increased dopamine levels, mirroring the effects of global anandamide presentation (Solinas, Justinova, Goldberg, & Tanda, 2006). This indicates that eCBs also influence dopamine levels in the brain, which may change impulsivity and decision-making. The interaction between the dopamine and eCB systems is the most likely source of cannabinoid-induced impulsivity as cannabinoid dysregulation appears to cause substantial changes in dopamine levels throughout the brain (see review in El Khoury, Gorgievski, Moutsimilli, Giros, & Tzavara, 2012).

Both THC and the synthetic cannabinoid WIN 55,212, however, have no effect on inhibitory control in the 5-CSRTT (Pattij et al., 2007; Wiskerke et al., 2011). The CB1R antagonist/inverse agonist rimonabant, however, dose-dependently improves baseline premature responses in the 5-CSRTT (Pattij et al., 2007). Pre-treatment with the CB1R antagonist O-2050 or rimonabant also dose-dependently reduced the inhibitory control deficits induced by amphetamine. A subsequent study revealed that rimonabant also reduced the inhibitory control deficits induced by nicotine (Wiskerke et al., 2012).

The 5-CSRTT and the IGT, including its equivalent animal model the rGT, examine different aspects of impulsivity. Impulsivity in human research is normally divided into three categories: motor (acting without forethought), attentional (lack of focus) and cognitive (difficulty considering the future over the present) (Bechara, Tranel, & Damasio, 2000; Malloy-Diniz, Fuentes, Leite, Correa, & Bechara, 2007). Bechara et al. (2000) demonstrated that the different forms of impulsivity are

neurologically distinct, although under normal circumstances it may be difficult to separate motor and cognitive impulsivity, since inhibiting a motor response might also cause the active intention to inhibit the response.

Based on Bechara et al.'s, (2000) categorization of impulsivity, the 5-CSRTT more closely reflects motor and attentional impulsivity, but not cognitive impulsivity. Premature responses in the 5-CSRTT are a good measure of motor impulsivity because they demonstrate whether or not the rat is considering the presence of the light before acting. The ability of the rat to respond correctly is a good measure of attentional impulsivity because the rats need to remain focused on the location of the light. Cocaine likely impairs attentional impulsivity as well as motor impulsivity in the 5-CSRTT unlike other dopamine increasing drugs that only affect motor impulsivity (van Gaalen et al., 2006). The 5-CSRTT does not, however, require any consideration of future responses as the reward size for a correct response is fixed.

The rGT contains the same measure of motor impulsivity as the 5-CSRTT, premature responses, but also measures cognitive impulsivity because the animals have to consider the overall number of rewards they will receive instead of simply choosing the first available option. Animal models of impulsivity are still relatively novel, and as such there are still some important gaps in the literature. The experiments described below sought to further elucidate the neurochemical basis of impulsivity and decision-making, and more specifically, examine the role of the eCB system in both baseline and dopamine receptor agonist-induced impulsivity.

The first experiment sought to examine how the eCB system was involved in motor and cognitive impulsivity. Previous tests of exogenous cannabinoids have shown little effect (Pattij et al., 2007; Wiskerke et al., 2011), but URB597 elicited some unique effects on dopamine such as a transient CB1 dependent spike in dopamine levels, and a slower rise in dopamine levels that was CB1 independent (Murillo-Rodríguez et al., 2011). Secondly, we wished to examine if the eCB system plays a role in cocaine-induced impulsivity in the 5-CSRTT. Examining if any aspect of cocaine-induced impulsivity is independent of the eCB system would further elucidate exactly what processes are CBR dependent. Finally, we wished to expand on the literature surrounding animal models of gambling behaviour by examining the role of the eCB system in the specific type of impulsivity tested in the rGT. The literature on humans indicates that the eCB system could play a vital role in cognitive impulsivity. Furthermore, the unique effects of cocaine on response accuracy in the 5-CSRTT warrants further examination of this drug in the rGT. Any behaviour elicited by cocaine and not amphetamine are likely related to differences in the neurochemical effects of these drugs. It was expected that URB597 would have no effect on the 5-CSRTT, but that THC and URB597 would induce a moderate effect on preference for the optimal choice in the rGT. It was also expected that cocaine-induced premature responding would be attenuated by rimonabant, and that cocaine would increase premature responding and lower the preference for the optimal choice in the rGT.

Method

Experiment 1: Effects of URB597 on visual attention and impulsivity in the 5-choice serial reaction time task

Subjects

Ten experimentally naïve male Sprague-Dawley rats (Charles River Laboratories, St. Constant, Quebec) weighing 316-368 g at the start of the experiment were used. Animals were single housed and maintained on a 12h:12h reverse light/dark cycle. Rats were handled once daily for several minutes for 5 days prior to the beginning of experimentation. This study was reviewed and approved by the Wilfrid Laurier University Animal Care Committee, and all experimental procedures were carried out in accordance with the Canadian Council on Animal Care Guide to the Care and Use of Laboratory Animals (CCAC, vol. 1, 1993).

Apparatus

Four identical modular test chambers (model ENV-007CT, Med Associates Inc., St. Albans, VT) were used. Each chamber contained a floor constructed of stainless steel bars, two cue lights, a house light, and a food cup connected to a food pellet dispenser with an infrared beam, which delivered 45 mg grain pellets (BioServ #F0165, Frenchtown NJ). The wall opposite the food cup contained five equally spaced 2.5 cm by 2.5 cm nose poke apertures located 1 cm above the floor (model ENV 115A, Med Associates Inc., Figure 1). Each aperture contained an LED and an infrared beam that registered head entry responses. Operant chambers were individually housed within sound-attenuating chambers fitted with a small fan to provide ventilation and masking noise. A video camera located within each

enclosure was used to monitor the animals. The test chambers were controlled by a digital interface (model DIG-716P2, Med Associates Inc.) connected to computer running custom software written in Med-PC (Version 4, Med Associates Inc.).

Drugs

Cocaine HCl (15 mg/kg, Sigma-Aldrich, Oakville, ON) was dissolved in 0.9% saline and injected IP in a volume of 1 ml/kg 5 min prior to placement into the testing chambers. URB597 (Cayman Chemicals, Ann Arbor, Michigan, USA) was added to a small amount of PEG 400 (Sigma-Aldrich, Oakville, Ontario, Canada) and saline solution. After mixing for several minutes, additional saline solution was added followed by a small amount of TWEEN 80 (polyoxyethylenesorbitan monooleate, ICN Biomedicals, Inc., Solon, OH). The final vehicle contained 0.5% TWEEN 80, 0.5% PEG 400 and 0.9% saline. URB597 was administered IP at a dose of 0.03, 0.3 and 1.0 mg/kg in a volume of 1 ml/kg 2 hours prior to testing.

Food Restriction

To maintain high motivation to perform the food-based reward task, *ad libitum* access to food was terminated and animals were placed on a mild food restriction regimen. Baseline weights were taken on the day food was removed, and daily food rations were provided to maintain 80%-90% *ad libitum* weight relative to baseline weights, adjusted for expected strain-specific growth as outlined on growth charts provided by the animal supplier. On the second, third and fourth food restriction days, animals were pre-exposed to the 45 mg pellets to reduce flavour neophobia. During the experiment animals were fed at least 1 hour following the completion of testing.

Habituation to the Test Apparatus

Habituation to the test apparatus began one week following the initiation of food restriction. All experimental procedures began approximately 1 hour into the dark cycle. On the first day of habituation rats were placed in the operant chambers for 20 min with the house lights, cue lights, and all five aperture lights illuminated. Two pellets were placed into each aperture and ten pellets were placed into the food cup to encourage exploration of the chamber. Rats consumed all pellets on the first day.

Nose-Poke Response Shaping

Following habituation, the nose poke response was shaped. First, both cue lights were illuminated and one non-contingent food pellet was delivered every minute into the food magazine. A nose poke into any of the five apertures also resulted in the delivery of a pellet and the illumination of the cue lights. A nose poke into the food magazine extinguished the cue lights until the next pellet was delivered. The session ended when 50 pellets were delivered, or 30 min had elapsed, whichever came first. The following day, the shaping procedure was modified such that the non-contingent pellet delivery was removed, and the aperture lights remained illuminated (i.e., active) for a maximum of 50 s. This procedure was repeated daily for each rat until 50 pellets were collected within the 30-min maximum session length. The following day, the training procedure was modified such that the session ended when rats collected a maximum of 100 pellets within a maximum 40-min session length.

5-CSRTT Training

Once a rat completed the final shaping session, 5-CSRTT training began. Animals were progressively trained through a series of seven programs (as decribed in Bari, Dalley, & Robbins, 2008,). Each session began with a free trial where a food pellet was delivered with the illumination of the house and cue lights. Head entry into the food magazine started the program, followed by the extinguishing of the cue lights and the activation of a 5 s ITI. At the end of the ITI a brief flash of light was randomly presented in one of the five apertures for a pre-determined stimulus duration (see Table 1). After the stimulus was presented a limited-hold period began during which a response into the previously illuminated aperture was reinforced by the presentation of a food pellet and the simultaneous illumination of cue lights. Cue lights remained illuminated until a head entry into the food cup was detected. This constituted a correct trial. A response into any of the non-illuminated apertures, which was recorded as an incorrect trial, resulted in a 5-s time-out period during which the house lights were extinguished. Failure to respond during the limited-hold period was recorded as an omission. A nose poke into any aperture prior to stimulus presentation was recorded as a premature response and resulted in a 5-s timeout. Repetitive nose pokes into any aperture outside of the ITI or limited-hold periods were recorded as perseverative responses, but had no specific consequences. Nose pokes and head entries into the food cups during the timeout periods were also recorded, but were devoid of consequences. Each training session continued until 120 trials were completed, or 60 min had elapsed, whichever came first. The difficulty of the program was increased by shortening the stimulus

presentation and limited-hold periods and changing criterions for progression (Table 1). The final training program had limited-hold and ITI lengths of 5 s, and a stimulus duration of 1 s. To advance to drug training rats had to achieve >60% accuracy and <20% omissions on the final session.

Procedure

Once rats completed training, drug testing began. The order of treatment was determined using a randomized block design. Rats received 0.03, 0.3, 1.0 mg/kg URB597, the URB597 vehicle, 15 mg/kg cocaine or saline on drug treatments days. Between drug treatments rats received at least one drug-free test session. Rats were required to achieve >60% accuracy and <20% omissions to progress to the next drug treatment. Drug-free sessions were repeated daily until the progression criteria were reached.

Experiment 2: Co-administration of cocaine and rimonabant

Subjects

The same rats were used from Experiment 1 once all drug treatments were completed.

Drugs

Cocaine was prepared in the same manner as in Experiment 1. Rimonabant was dissolved into ethanol, mixed with 1% TWEEN 80, and the ethanol was subsequently evaporated under a stream of nitrogen gas. Saline was then added and the solution was mixed until the drug and TWEEN 80 were well dispersed. The final vehicle contained 1% TWEEN 80 and 0.9% saline. Rimonabant (1.0 mg/kg) or its vehicle were administered IP in a volume of 1 ml/kg body weight 30 min prior to

the session, and cocaine (15 mg/kg) or its vehicle were administered 5 min prior to the session. The order of treatments was determined using a randomized block design.

Apparatus and Procedure

The apparatus and procedure were identical to Experiment 1.

Experiment 3: The effect of URB597 on impulsivity and decision-making in the rodent Gambling Task

Subjects

Thirteen experimentally naïve Sprague-Dawley rats weighing between 300-350 g at the start of the experiment were used. Animals were single housed and maintained on a reverse 12h:12h light/dark cycle. Rats were briefly handled once or twice daily for 5 days prior to the beginning of experimentation. Experimentation began one hour into the dark cycle. This study was reviewed and approved by the Wilfrid Laurier University Animal Care Committee, and all experimental procedures were carried out in accordance with the Canadian Council on Animal Care Guide to the Care and Use of Laboratory Animals (CCAC, vol. 1, 1993).

Apparatus

The apparatus was identical to that used in Experiments 1 and 2.

Drugs

Cocaine and URB597 were prepared in the same manner as in Experiment 1. THC (THC Pharm GmbH, Frankfurt) was dissolved into ethanol, mixed with a small quantity of TWEEN 80, and the ethanol was subsequently evaporated under a

stream of nitrogen gas. The drug was then suspended in saline solution. The final vehicle contained 1% TWEEN 80 and 0.9% saline. THC was administered IP at a dose of 1.0 or 1.5 mg/kg in a volume of 1 ml/kg body weight, 30 min prior to testing.

Food Restriction

Food restriction was conducted in the same manner as in Experiments 1 and 2.

Habituation

Habituation was conducted in the same manner as in Experiment 1.

Shaping and 5-CSRTT Training

Shaping and training were identical to that used in Experiment 1, up to training stage 1 of the 5-CSRTT training programs (see Table 1). rGT training began once rats reached >80% accuracy and <20% omissions.

rGT Training

Experimentation began 1 hour into the dark cycle. Rats were randomly assigned to either the 'A' or 'B' versions of the program. Each session lasted 30 minutes. For all A programs, a nose poke into the first aperture resulted in a 90% chance of obtaining one food pellet and a 10% chance of a 5 s timeout (P2), a nose poke into the second aperture had a 40% chance of obtaining four pellets and a 60% chance of a 40 s timeout (P4), a nose poke into the fourth aperture had an 80% chance of producing two pellets and a 20% chance of a 10 s time out (P1), and the fifth aperture had a 50% chance of producing three pellets and a 50% chance of a 30 s timeout (P3)(Zeeb et al., 2013). The names P1, P2, P3, and P4 refer to the most to least favourable options in terms of maximum total number of pellets that could be earned, with P1 being the optimal choice (see Table 2). The B programs had the

probabilities associated with each aperture reversed, with the centre aperture remaining unused in both programs (i.e., nose pokes into the centre aperture were not recorded and had no consequences). All rats first went through 7 days of fixed response training. In the fixed response training, rats initiated a trial with a nose poke into the food cup, which began a 5 s ITI similar to the 5-CSRTT. If the rat responded during the ITI the response was recorded as a premature response and the rat was punished with a 10 s time out. An aperture was then randomly illuminated for each trial, similar to the 5-CSRTT. The stimulus remained illuminated for 10 s. If the rat responded correctly, they received either the associated reward or the house lights were extinguished to begin the time out period. If the rat did not respond within this time, it was recorded as an omission and a new trial was initiated following a head entry into the food cup. Following collection of the reward or the end of the timeout period, a 5 s ITI occurred after which a new trial was initiated with a head entry into the food cup. Repeated responses into the same aperture were recorded as perseverative responses. A randomization without replacement procedure was used to ensure equal presentation of all apertures. After a week of fixed response training all rats were moved on to the full rGT program. The full program was identical to the fixed response program except instead of one aperture being illuminated randomly following the initiation of a trial, all four apertures were illuminated and the rats could choose one option for each trial. After the rat had chosen, all of the aperture lights were extinguished followed by either delivery of the associated reward or the beginning of the timeout period. Rats advanced to drug training when the number of

responses into P1 (the optimal choice) was higher than the other three available choices for 3 consecutive days. Rats received drug treatments in a randomized order followed by a wash-out day to ensure that there was no interaction between drug treatments. The next drug trial was then administered the day following the wash-out.

Experiment 4: Vehicle Test

As it appeared that the URB597 vehicle may have affected choice behaviour (results shown below), an additional experiment was conducted to compare the URB597 vehicle to saline in the rGT using the seven rats from Experiment 3 that were still responding optimally.

Results

Experiment 1: Effects of URB597 on visual attention and impulsivity in the 5-choice serial reaction time task

The mean percentage correct responses across treatments for cocaine, URB597, and vehicle control treatments are shown in Figure 2. A planned contrast comparing the cocaine treatment to its saline vehicle revealed that cocaine administration significantly decreased the percentage of correct responses relative to the saline ($F_{1,9}$=24.075, p=.001), but URB597 had no effect on the percentage of correct responses (p>.05). Cocaine exposure also significantly increased premature responses ($F_{1,9}$=13.626, p=.005) and timeout responses ($F_{1,9}$=7.585, p=.033), but not omissions or perseverative responses (Figure 3). URB597 had no significant effects on premature responses, timeout responses, omissions, or perseverative responses

(Figure 3). The latency to respond and collect rewards across treatments is shown in Figure 4. Cocaine significantly decreased latency to correct responses ($F_{1,9}$=14.742, p=.009) and latency to collect ($F_{1,9}$=8.397, p=.027), but not latency to incorrect responses. URB597 had no significant effects on response latencies (p>.05).

Experiment 2: Co-administration of cocaine and rimonabant

The mean percent correct responses for rimonabant and cocaine across treatments can be seen in Figure 5, and premature responses across treatments can be seen in Figure 6. As expected, cocaine treatments significantly lowered correct responses ($F_{1,9}$=70.359, p<.001) and increased premature responses ($F_{1,9}$=33.575, p<.001). However, rimonabant had no effect on correct responses or premature responding (p>.05). There was also no interaction, indicating that the effects of cocaine were not attenuated by rimonabant.

Experiment 3: The effect of URB597 on impulsivity and decision-making in the rodent Gambling Task

Acquisition

Once rats consistently preferred the optimal choice over the other three options and moved onto drug training, their data from wash-out days were included in the acquisition graphs to demonstrate that optimal choice preference was maintained overall. Four rats never reached the optimal choice preference criteria and were not included in the analysis, bringing the total number of rats to nine. The means of the percent choice for each option across days can be seen in Figure 7. An arcsine transformation was used to normalize all choice preference data prior to analysis.

As can be seen, the percent choice for each option significantly changed over time ($F_{12,96}$=2.124, p=.022). Rats also responded significantly differently on each aperture, indicating they were able to differentiate the properties of each choice ($F_{3,24}$=8.228, p=.001). The preferred aperture changed over time indicating a significant progression towards optimal choice preference ($F_{36,288}$=2.105, p<.001). On day 7 rats showed a significant preference for the optimal choice ($F_{3,32}$=7.796, p<.001), and picked the optimal choice (P1) significantly more often than P2 (p=.015), P3 (p<.001), and P4 (p=.011).

Drug Data

All data were examined in two manners: with all rats included in the analysis, and excluding rats that did not prefer the optimal choice on vehicle days. The mean percent choice across treatments for all rats can be seen in Figure 8, and the mean percent choice excluding rats that did not prefer the optimal choice on the vehicle day can be seen in Figure 9. An arcsine transformation was used to normalize all choice preference data. Rats maintained the optimal choice preference across treatments ($F_{3,33}$=7.718, p<.001), but there was no effect of URB597 (p>.05). Removing the rats that did not prefer the optimal choice during the vehicle control did not alter the optimal choice preference ($F_{3,18}$=15.184, p<.001), but URB597 still had no significant effect (p>.05). Rats maintained the optimal choice preference across all THC treatments ($F_{2,36}$=7.732, p<.001), but THC had no significant effect on responding (p>.05). Removing the non-optimal vehicle rat data maintained the optimal choice preference ($F_{2,21}$=11.372, p<.001), but there was still no main effect of THC (p>.05). Rats maintained the optimal choice preference in the saline and

cocaine treatments ($F_{1,36}$=10.584, p<.001), but cocaine had no effect on responding (p>.05). Excluding rats that did not prefer the optimal choice during the saline control maintained optimal choice preference ($F_{1,24}$=10.102, p<.001), but the effect of cocaine remained non-significant (p>.05).

The number of premature responses and total response latency across treatments can be seen in Figures 10 and 11, respectively. Administration of URB597 produced a non-significant trend towards increasing premature responding ($F_{3,27}$=2.847, p=.051), but no dose of URB597 was significantly different from the vehicle control (p>.05). URB597 also had no effect on latency (p>.05). Neither THC nor cocaine had any significant effect on premature responses (p>.05) or latency (p>.05).

Experiment 4: Vehicle Test

The mean percentage correct responses of saline and the UR597 vehicle can be seen in Figure 12. These data show that the URB597 vehicle did significantly alter responding ($F_{1,6}$=9.2, p=.023). There was, however, no difference between treatments for each individual choice (p>.05).

Discussion

The first experiment suggests that URB597, like THC and WIN 55,212, has no effect on impulsive action. Previous experiments have demonstrated that THC and WIN 55,212 have no effect on the two main measures of impulsivity in the 5-CSRTT–premature responding and percent correct responding–but increased response and

collection latency (Pattij et al., 2007; Wiskerke et al., 2011). URB597, like THC and WIN 55,212, did not affect premature responding or percent correct responding, but unlike THC and WIN 55,212, it did not affect latency. Cocaine also generally affected performance as expected, increasing premature responding, increasing timeout responding, decreasing correct response latency, latency to collect and decreasing percent correct responding.

It is not surprising that URB597 had no effect on premature responding or correct responses in the 5-CSRTT, but it is interesting, however, that URB597 had no effect on latency, as previous studies using cannabinoid receptor agonists found increased response and collection latency in the 5-CSRTT (Pattij et al., 2007; Wiskerke et al., 2011). The increase in latency induced by other cannabinoids is likely the result of locomotor suppression, given that exogenous cannabinoid receptor agonists such as THC have been consistently found to decrease locomotor activity (Herkenham, 1992). Anandamide administration by itself also decreases locomotor activity similar to exogenous cannabinoids (de Lago, de Miguel, Lastres-Becker, Ramos, & Fernández-Ruiz, 2004; Romero et al., 1995). URB597, however, does not affect locomotor activity, which likely explains why it had no effect on response or collection latency (Adamczyk et al., 2009; Jayamanne et al., 2006).

The finding that cocaine increased premature responses, increased timeout responses, decreased correct responses, and decreased correct response and collection latency, directly replicated results from previous literature (van Gaalen et al., 2006). Although cocaine did not affect incorrect response latency, it was likely because baseline incorrect response latency was low enough that there was a floor

effect. Cocaine was therefore an effective positive control for validating the methods of the study.

The results of the second experiment did not confirm our hypothesis that cocaine induced-impulsivity would be attenuated by rimonabant. Baseline premature responding was also not improved by rimonabant (Figure 6). The results did, however, demonstrate that cocaine increased impulsivity in a manner similar to that observed in Experiment 1 (Figure 3). It is noteworthy that tolerance did not develop to this effect of cocaine across two experiments. The increase in premature responding and diminished correct responses by cocaine were also not attenuated by rimonabant, in contrast with studies using other psychomotor stimulants (Pattij et al., 2007; Wiskerke et al., 2011, 2012).

Previous work comparing several stimulants on measures of impulsivity showed some unique results with cocaine (van Gaalen et al., 2006). It is possible that, unlike the other drugs examined, the neural mechanisms underlying cocaine-induced impulsivity appear to be independent of the eCB system. Previous studies have demonstrated that pretreatment with rimonabant has no effect on cocaine self-administration, reinforcement or discrimination, but significantly reduced sensitization and relapse (Filip et al., 2006). A 3.0 mg/kg dose of rimonabant completely blocks the effects of CB1R agonists (Pério et al., 1996; Rinaldi-Carmona et al., 1994). Thus, it is unlikely that the lack of attenuation by rimonabant in the present study was caused by an insufficient dose of rimonabant. These findings suggest that some of the effects of cocaine are CB1-dependent while others are not. If this is true it would represent a very interesting previously unknown difference

between cocaine and other stimulants that would help further elucidate our understanding of the interaction between the dopamine and eCB systems.

It is possible, however, that the results of the second experiment were simply due to the rats not being experimentally-naïve, given that there was also no reduction of baseline premature responding by rimonabant. That is, it is possible that previous exposure to the study's methods caused low premature responding such that the methods employed were no longer sensitive enough to detect any effect of rimonabant. The rats used in this experiment had also been previously exposed to cocaine, but not rimonabant. Rats may have developed tolerance to some of the behavioural effects of cocaine, but not the polydrug effect of rimonabant, resulting in no attenuation of the effect of cocaine.

Lastly, a clear preference for the optimal choice was found after approximately one week of training, which is consistent with previous experiments on the rGT (Zeeb et al., 2009). Contrary to our hypothesis, however, no treatment had any effect in the rGT. URB597 and THC had no effect on optimal choice preference or premature responding in the rGT. Cocaine also did not alter optimal choice preference or increase premature responding. The results of the last experiment also contrasts with existing literature as previous experiments have demonstrated that amphetamine consistently lowers optimal choice preference and increases premature responding on the rGT (Zeeb et al., 2009, 2013). Cocaine and amphetamine have different modes of action on dopamine in the brain. Cocaine binds to the dopamine transporter (DAT) to slow reuptake of dopamine into the synapse (Beuming et al., 2008), but amphetamine enters the neurons and effects

upstream processes that result in the phosphorylation of DAT, which ceases dopamine transport (Miller, 2012). This difference in action is a possible source of the differential findings between amphetamine and cocaine.

The vehicle test was conducted to ensure that the URB597 vehicle had no effect on responding. The test initially revealed that there was a difference between saline and the vehicle, but when each option in the rGT was compared individually there was no difference between saline and the URB597 vehicle. The effect shown, however, indicates that the URB597 vehicle was improving responding, which is the opposite of that observed in the initial rGT tests.

The results of the final experiment using the rGT found no effect of URB597, THC or cocaine. It is possible that the processes required to complete the rGT are completely independent of the effect of these drugs, but given the complexity of the rGT this is unlikely. If, however, the drugs truly have no effect, it would again speak to how the behavioural effect of cocaine differs from amphetamine and other drugs that increase dopamine.

A larger sample size would increase power and might reveal a significant effect that was not found in the current study as previous studies have used larger sample sizes when testing the rGT (Zeeb et al., 2009, 2013). The cocaine data show a clear trend at reducing optimal choice preference and increasing preference for the other options, but the difference in choice across treatments did not reach the criteria for significance.

The data also show a trend towards the low dose of THC increasing latency to respond. Unlike the 5-CSRTT, latency to respond was not separated into two

different categories. Correct response latency in the 5-CSRTT examines the time required to process the information of the stimulus presentation, while incorrect response latency measures the time required for the animal to choose any aperture. In the rGT, rats are presented with all options at once and are given the opportunity to choose, unlike in the 5-CSRTT, where there is only one response that will be rewarded. Latency in the rGT measures the time required for the rat to make a decision out of four possible options, not simply respond correctly. Latency to respond in each task therefore represents unique phenomena, and this may account for differences in the observed results. It is possible, however, that examining latency for the optimal choice and non-optimal choices separately would reveal exactly how THC affects latency in the rGT..

Despite rats choosing the optimal choice more often than the others in the final rGT test, several rats began choosing the P3, or 50% option, again by the end of the experiment. Previous tests of the rGT have used fewer treatments and included a third day off where the rats remained out of the boxes between wash out and the administration of the new treatment (Zeeb et al., 2013). It is possible that in order to not be tempted by larger rewards rats require a day where they are not exposed to the task. Animals in our experiment were also single housed which was previously demonstrated to cause subjects to be more tempted by large rewards versus pair-housed and environmentally-enriched rats (Zeeb et al., 2013).

Additionally, the order of options in the two versions of the rGT were simply mirror images of each other. This procedure was included to control for potential position biases, but it did not control for a general preference for the options

presented in the first aperture and the last aperture versus the centre two apertures. The most common choice among rats that did not achieve optimal choice was the 50% option, which was presented in the last aperture in the A version of the task and the first aperture in the B version. Preference for the 50% option may have been the result of side preference instead of the rats actually seeking a larger reward. A future version of the experiment should control for general side preference by ensuring the order of the options is rearranged, not simply mirrored, to created the second version of the rGT.

The results of these experiments demonstrate that acute administration of cannabinoid receptor agonists have no effect on impulsivity. Despite URB597 administration more closely modeling a natural increase of anandamide in the brain, it produced a similar effect to previously examined cannabinoid agonists by having no effect on measures of impulsivity in the 5-CSRTT and the rGT. We also further demonstrated that cocaine might have some unique effects not present in other stimulants that require further research to fully understand. A future test of the rGT should examine how chronic administration of THC could alter optimal choice responding over time since the human work suggests that impaired decision-making by CB1 agonists may require chronic administration. Taken together, the results of the present study add to the growing knowledge about impulsivity and decision-making that may aid in understanding impulsive behaviour and problem gambling.

Figures

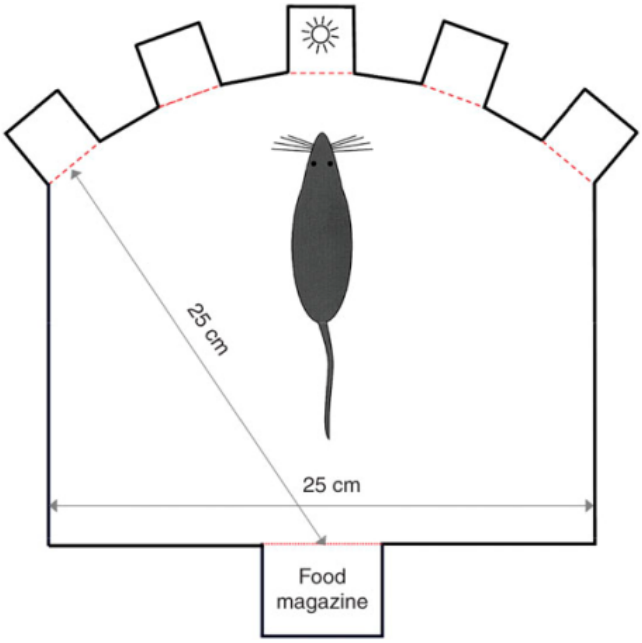

Figure 1. Schematic diagram of test apparatus used in the 5-CSRTT. Each of the five equidistant nose-poke apertures contained an infrared beam to detect head entries, and an LED used to signal active apertures. Two cue lights located on either side of the food magazine indicated the presentation of a reward when turned on, and marked the beginning of the ITI period when turned off (image reproduced from Bari et al., 2008).

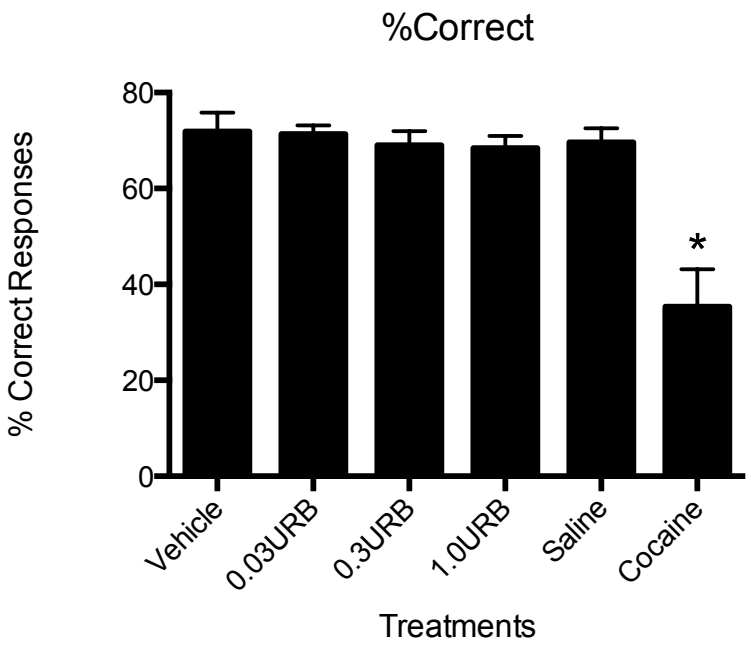

Figure 2. Percentage (+SEM) of correct responses in the 5-choice serial reaction time task. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine. *significantly different from vehicle control, $p<0.05$.

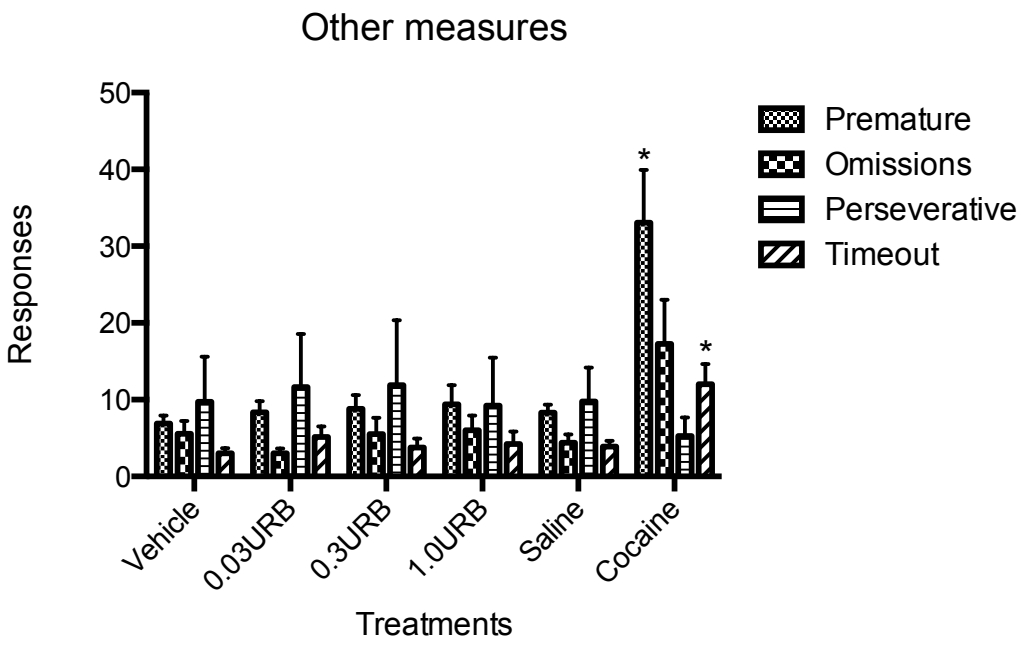

Figure 3. Number (+SEM) of premature responses , omissions, perseverative responses and timeout responses in the 5-choice serial reaction time task. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine. Omissions are the total number of trials without response prior to the limited hold period; Premature responses are the total number of premature responses performed after trial initiation but before presentation of the light stimulus; Perseverative responses are total nose-pokes into the unlit apertures following the presentation of a reward, but before the initiation of the next ITI period; Timeout responses total number of responses performed during a timeout period. *significantly different from vehicle control $p<0.05$.

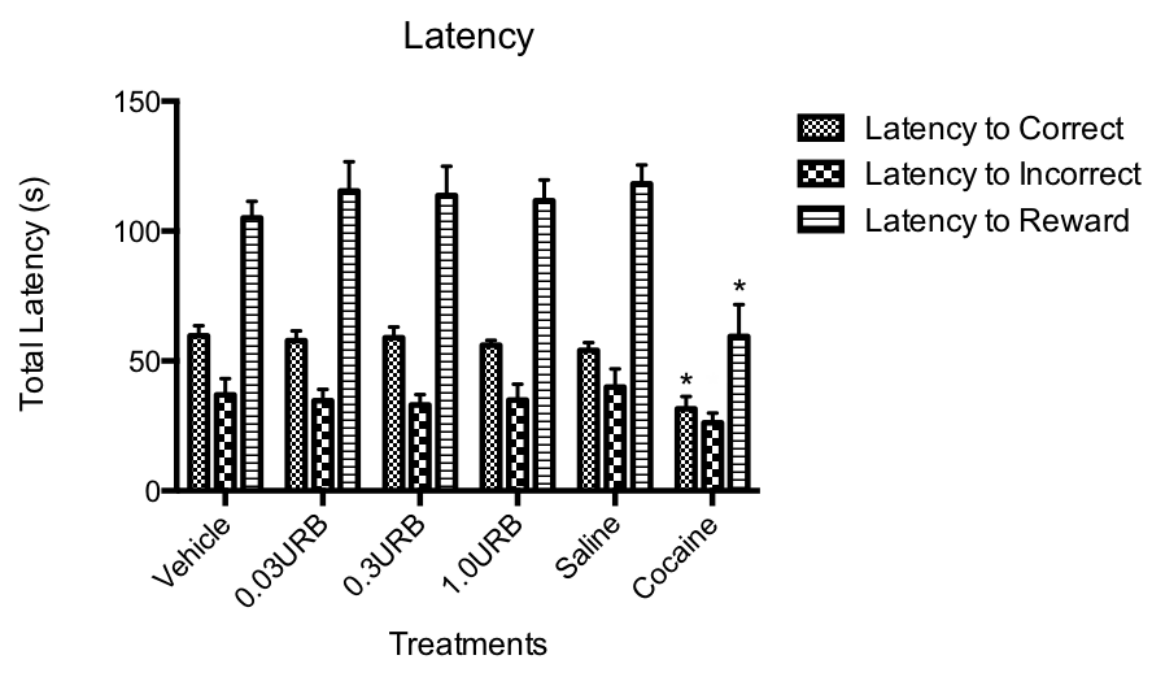

Figure 4 The latency (s+SEM) to respond correctly, to respond incorrectly and to collect the food reward in the 5-choice serial reaction time task. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine. Latency to Correct Response is the summation of time from the onset of the light stimulus to the performance of a correct nose poke response; Latency to Incorrect Response is the summation of time from the onset of the light stimulus to the performance of a incorrect nose poke response; Latency to Retrieve Reward is the summation of time from the performance of a correct response to the retrieval of the food reward from the magazine. *significantly different from vehicle control $p<0.05$.

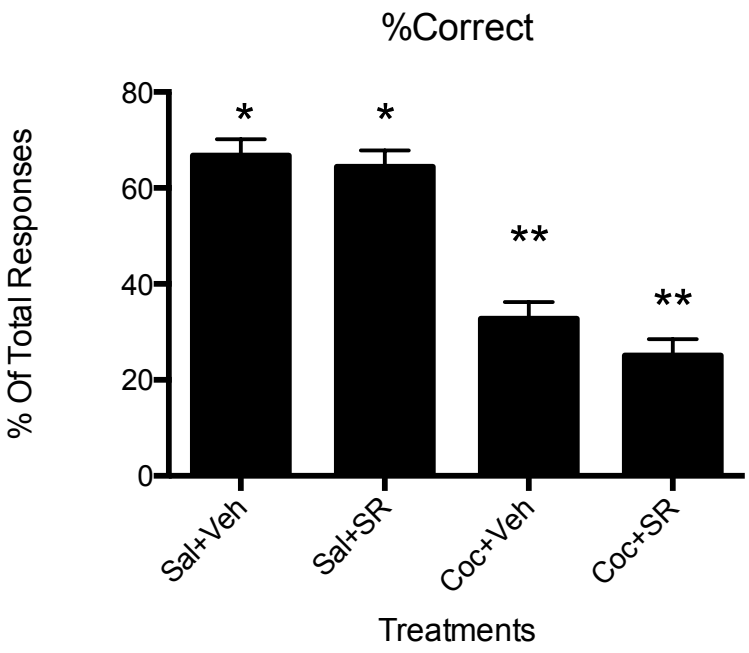

Figure 5 The percentage (+SEM) of correct responses in the 5-choice serial reaction time task. Sal= vehicle for cocaine; Veh= vehicle for rimonabant; SR= 3.0 mg/kg rimonabant; Coc= 15 mg/kg cocaine. Each bar with * is significantly different from each bar with ** $p<0.05$.

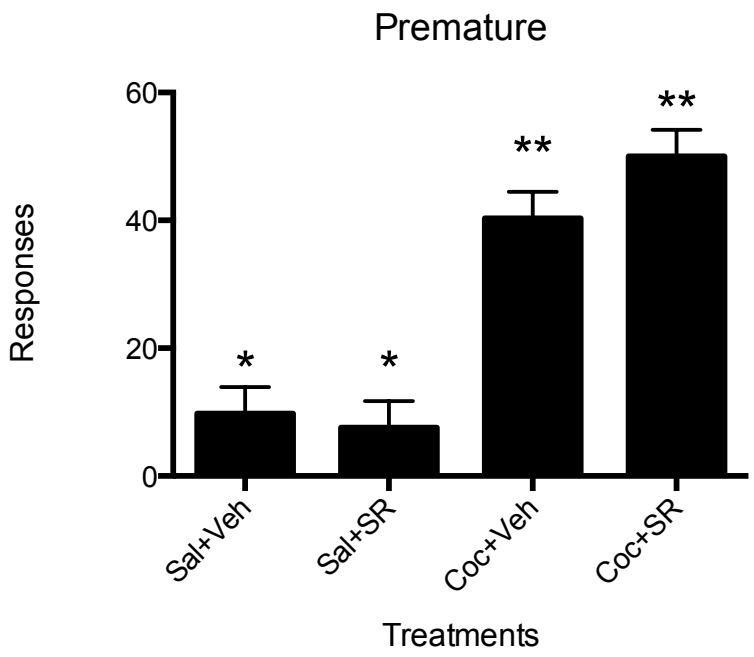

Figure 6 The mean (+SEM) number of premature responses by rats in a session. Sal= vehicle for cocaine; Veh= vehicle for rimonabant; SR= 3.0 mg/kg rimonabant; Coc= 15 mg/kg cocaine. Each bar with * is significantly different from each bar with ** $p<0.05$.

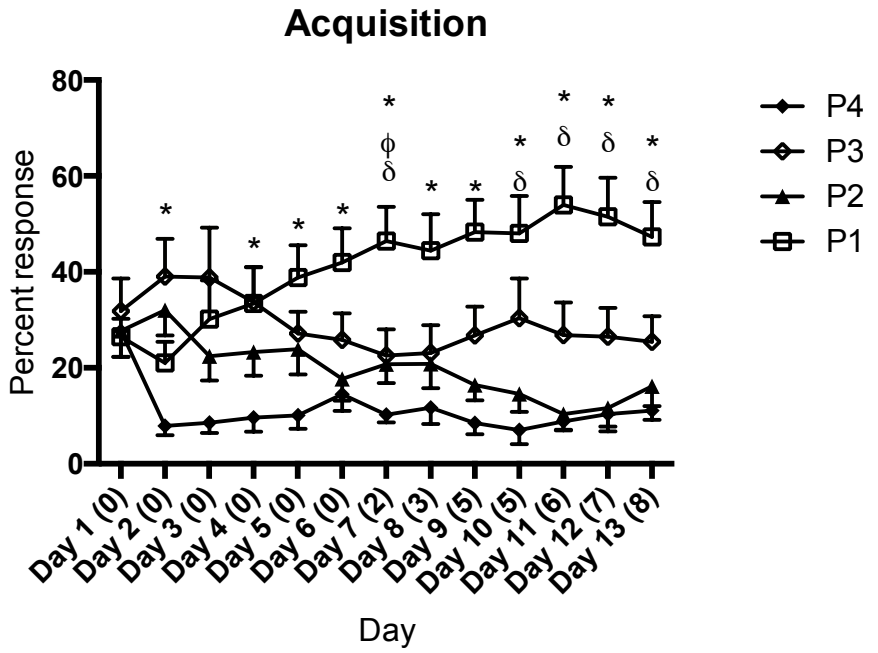

Figure 7 Mean (+SEM) percent choice for each option over acquisition days. The number in brackets refers to the number of rats that have progressed to drug training. Washout data was used for these rats after this point. P1= 80% chance of two pellets or a 20% of a 10s timeout; P2= 90% chance of one pellet or a 10% chance of a 5s timeout; P3= 50% chance of 3 pellets or a 50% chance of a 30s timeout; P4= 40% chance of four pellets a 60% chance of a 40s timeout. * indicates the optimal choice (80%) is significantly different from the 40% option ($p<.0167$). Φ indicates the optimal choice (80%) is significantly different from the 90% option ($p<.0167$). δ indicates the optimal choice (80%) is significantly different from the 50% option ($p<.0167$).

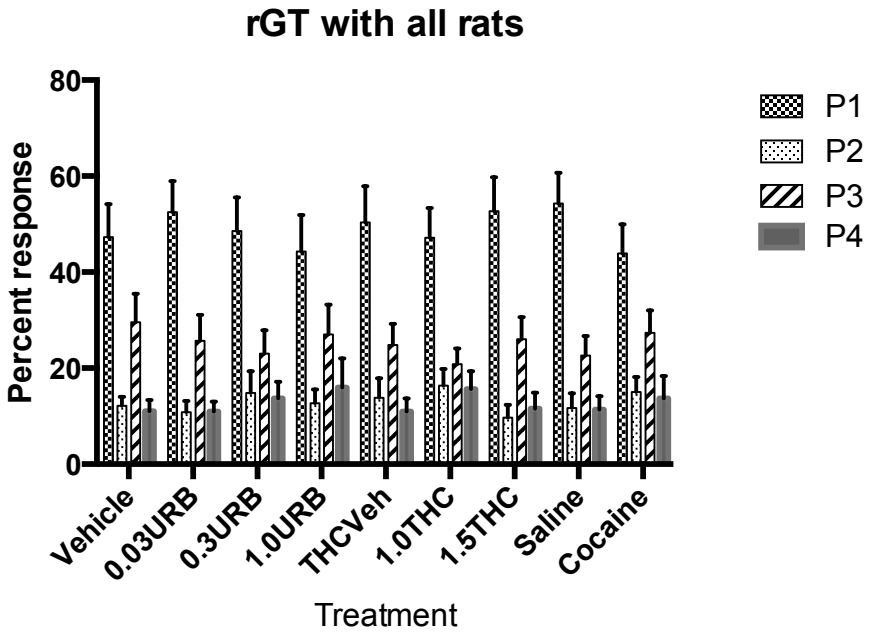

Figure 8 The mean (+SEM) percentage responses for each of the options available to rats in the rGT across treatments. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; THCVeh=vehicle for THC; 1.0THC= 1.0 mg/kg Δ^9-tetrahydrocannabinol; 1.5THC= 1.5 mg/kg Δ^9-tetrahydrocannabinol; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine; P1= 80% chance of two pellets or a 20% of a 10s timeout; P2= 90% chance of one pellet or a 10% chance of a 5s timeout; P3= 50% chance of 3 pellets or a 50% chance of a 30s timeout; P4= 40% chance of four pellets or a 60% chance of a 40s timeout.

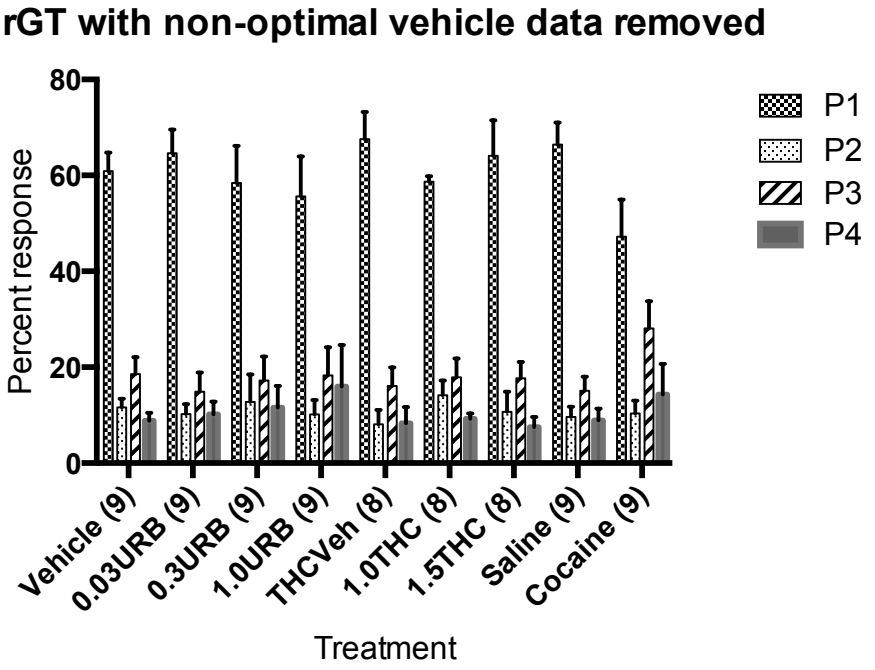

Figure 9 The mean percentage choice (+SEM) for each of the options available to rats during a session in the rGT across treatments. If a rat did not have optimal choice preference during a vehicle control, the data was removed from the specific comparison. The number in brackets represents the number of subjects left in the analysis. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; THCVeh=vehicle for THC; 1.0THC= 1.0 mg/kg Δ^9-tetrahydrocannabinol; 1.5THC= 1.5 mg/kg Δ^9-tetrahydrocannabinol; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine; P1= 80% chance of two pellets or a 20% of a 10s timeout; P2= 90% chance of one pellet or a 10% chance of a 5s timeout; P3= 50% chance of 3 pellets or a 50% chance of a 30s timeout; P4= 40% chance of four pellets or a 60% chance of a 40s timeout.

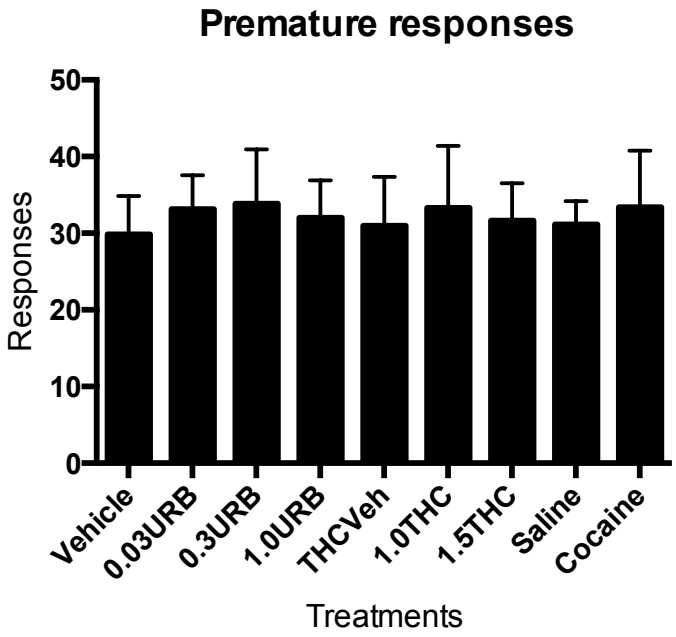

Figure 10 The mean number of premature (+SEM) responses in the rGT by rats across treatments. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; THCVeh=vehicle for THC; 1.0THC= 1.0 mg/kg Δ^9-tetrahydrocannabinol; 1.5THC= 1.5 mg/kg Δ^9-tetrahydrocannabinol; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine.

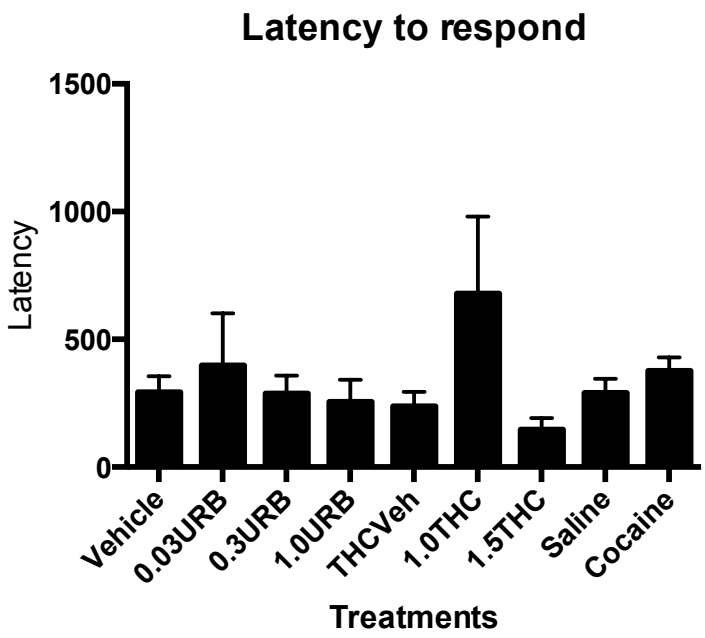

Figure 11 The latency (s+SEM) to respond across treatments. Vehicle=vehicle for URB597; 0.03URB=0.03 mg/kg URB597, 0.3URB=0.3 mg/kg URB597; 1.0URB= 1.0 mg/kg URB597; THCVeh=vehicle for THC; 1.0THC= 1.0 mg/kg Δ^9-tetrahydrocannabinol; 1.5THC= 1.5 mg/kg Δ^9-tetrahydrocannabinol; saline=vehicle for cocaine; cocaine= 15 mg/kg cocaine.

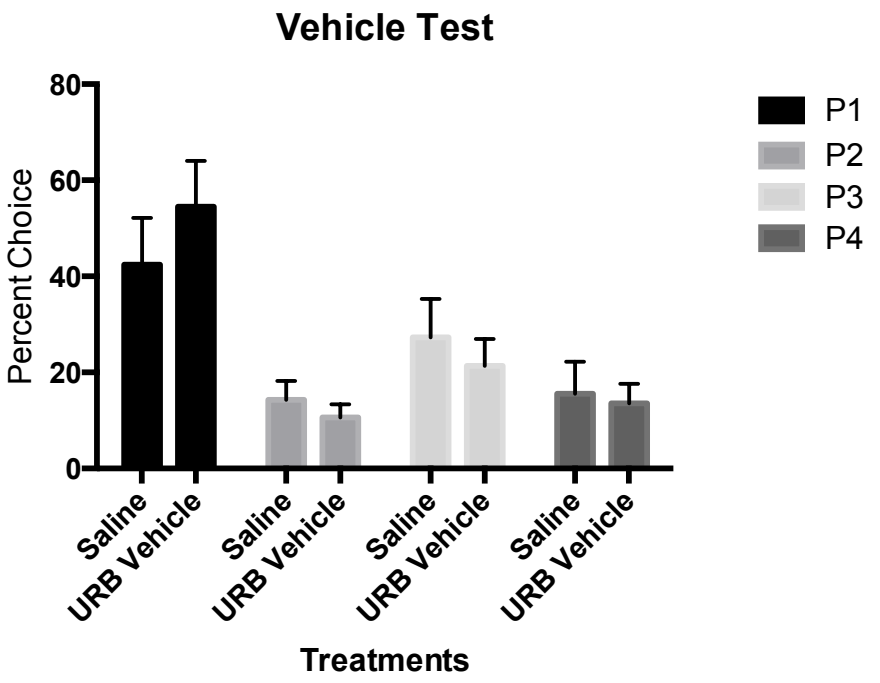

Figure 12 The mean percentage choice for each of the options available to rats during a session in the rGT across treatments. Saline= cocaine vehicle; P1= 80% chance of two pellets or a 20% of a 10s timeout; P2= 90% chance of one pellet or a 10% chance of a 5s timeout; P3= 50% chance of 3 pellets or a 50% chance of a 30s timeout; P4= 40% chance of four pellets or a 60% chance of a 40s timeout.

Tables

Table 1. Training parameters across 5-CSRTT training stages.

Training Stage	Stimulus Duration (s)	ITI (s)	Limited Hold (s)	Criterion for progression
1	30	2	30	≥ 30 Correct Trials
2	20	2	20	≥ 30 Correct Trials
3	10	5	10	≥ 50 Correct Trials
4	5	5	5	≥ 50 Correct Trials > 80% Accuracy
5	2.5	5	5	≥ 50 Correct Trials > 80% Accuracy < 20 % Omissions
6	1.25	5	5	≥ 50 Correct Trials > 80% Accuracy < 20 % Omissions
7	1	5	5	≥ 50 Correct Trials > 60% Accuracy < 20 % Omissions
Testing	1	5	5	≥ 1 wash out day > 60% Accuracy < 20 % Omissions

Table 2. Hypothetical maximum number of pellets for each option

Name	Probability of trial being rewarded	Number of pellets	Time out when trial is not rewarded (s)	Hypothetical maximum number of pellets
P1	**80%**	**2**	**10**	**411**
P2	**90%**	**1**	**5**	**295**
P3	**50%**	**3**	**30**	**135**
P4	**40%**	**4**	**40**	**99**

Assuming no premature responses or omissions and a 5 second trial time

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