SALIENT WIN-PAIRED CUES MEDIATE DECISION MAKING ON A RODENT GAMBLING TASK

by

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Abstract

Background

Pathological gambling is a pervasive and destructive behavioral disorder in which individuals lose control over their gambling behavior, leading to severe personal, social and financial consequences. Current animal models of gambling behavior such as the rodent gambling task (rGT) are useful tools with which to evaluate choice behavior. However, they are limited in their insights into gambling behavior in that they mostly model dimensions of economic decision-making, but not the salient cues intrinsic to human gambling paradigms. Here, we developed a task called the cued rGT to examine the potential influence of salient win-associated cues on decision-making.

Methods

16 male Long-Evans rats were tested on either the traditional or a cued version of the rGT. Once trained, they were treated with a number of dopaminergic compounds to delineate the role of this neurotransmitter in guiding choice behavior in both cued and uncued tasks.

Results

Animals on the cued task showed a more disadvantageous choice preference at baseline than animals on the uncued task. Amphetamine caused a significant increase of a safe, certain option in both versions of the task, a result that is somewhat consistent with past findings. Quinpirole, a D2-like agonist, increased disadvantageous choice in the cued group but not the uncued group. There were no effects of eticlopride, a D_2-like antagonist, or selective D4 drugs on choice performance.

Conclusions

Salient win-associated cues are sufficient to drive a shift towards disadvantageous choice

preference. This effect appears to be mediated, at least in part, by D2-like receptors. These finding suggest the cued rGT is a valuable model with which to study how salient cues can invigorate maladaptive decision making, an important and understudied component of pathological gambling and substance use disorders.

Preface

This manuscript was conceived and designed by Michael M Barrus and Dr Catharine Winstanley. Experimental work was carried out by Michael M Barrus. The manuscript was written by Michael M Barrus with supervision and editorial oversight from Dr Catharine Winstanley. Jay G Hosking provided additional input. All experimental work was performed under ethics certificate A13-0011 “Role of salient cues in guiding rodent decision making”, which was approved by the University of British Columbia's Animal Care Committee. Husbandry was performed in accordance with the standards set forth by the Canadian Council of Animal Care.

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Introduction

Gambling is a common and widespread form of entertainment that is often relatively innocuous but can lead to considerable distress for some individuals. While most people are able to gamble within reasonable limits, some individuals develop compulsive and maladaptive gambling behaviours. In particularly severe cases, these behaviours meet the criteria for Pathological Gambling (PG), a DSM-V recognized diagnosis that bears resemblance to substance addiction (Potenza 2006). Estimates suggest around 12.5% of the general population demonstrates sub-clinical problem gambling, and 2.5% meet the criteria for pathological gambling (Cunningham-Williams et al 2005). The criteria for PG includes "a preoccupation with gambling... a need to gamble with increasing amounts of money in order to achieve the desired level of excitement... chasing one's losses" and "(committing) illegal acts such as forgery, fraud, theft or embezzlement to finance gambling" (Reilly 2013). The prognosis for an individual diagnosed with PG is unfavorable; criminality and massive debt are common amongst problem gamblers and suicide rates are as high as 24% (DeCaria et al 1996). The neurobiological underpinnings of the disorder are not clear, and a better understanding of the neuropathology of PG would be enormously helpful in developing effective therapeutic interventions and improving the long-term prospects of those diagnosed as pathological gamblers (Madden et al 2007).

The neurotransmitter dopamine has attracted interest for its potential role in PG and other forms of disordered behaviour. The two major dopamine pathways are the nigrostriatal pathway and the mesolimbic pathway, both emerging from the midbrain (Kandel et al 2000). Projections from the substantia nigra comprise the nigrostriatial pathway, which is critical in coordinating movement. Loss of neurons in this region leads to motor disorders, such as those seen in Parkinson’s disease. The mesolimbic pathway projects from the ventral tegmentum to limbic regions and the forebrain, and is thought to be critical for the development and maintenance of

addictive disorders. There are five known subtypes of dopamine receptors, classed into two families. D_1-like receptors (D_1 and D_5) are excitatory and distributed widely throughout the cortex, striatum, nucleus accumbens, amygdala and substantia nigra, while occurring to a lesser extent in other regions. D_2-like receptors (D_2, D_3, D_4) are inhibitory, and $D_{2/3}$ receptors are primarily expressed in the striatum and the nucleus accumbens, but also occur in limbic regions and the midbrain. D_4 receptors have a more limited range and are primarily distributed in areas of frontal cortex and limbic structures (Missale et al 1998). D_1 receptors are exclusively post-synaptic, while $D_{2/3}$ receptors can be expressed both pre and post synaptically. These presynaptic autoreceptors serve an important regulatory function, as they play a role in the negative feedback systems that regulate production, synthesis and firing rate of dopamingergic neurons (Missale et al 1998). These autoreceptors are primarily associated with dopaminergic neurons in the ventral tegmental area, though they have also been identified in the nucleus accumbens and other mesolimbic regions (Wolf et al 1990). The D_2-like receptors have attracted particular attention as therapeutic targets for disorders ranging from schizophrenia to addiction (Kandel et al 2000), and more recently, pathological gambling (Comings et al 1996).

Dopamine and pathological gambling

Interest in the relationship between PG and dopamine increased when the disorder appeared in Parkinson's disease patients taking dopamine agonists to control their motor symptoms. These individuals, who had not previously engaged in problem gambling, developed uncontrollable gambling behaviours shortly after beginning treatment with dopamine agonists that primarily acted on D_2-like receptors (Dodd et al 2005). Patients reported that discontinuing their medications resulted in the cessation of gambling behaviours and the urge to gamble. The tight temporal relationship between the administration of dopamine agonists and the PG-like

behaviour suggested a causal relationship between dopamine and problem gambling, and subsequent research seems to support this notion (Dodd et al 2005). Researchers have since demonstrated that amphetamine (a psychostimulant that increases dopamine presence in the synapse through enhanced release and reuptake blockade, among other mechanisms of action) increases the urge to gamble in problem gamblers and increases the speed at which they read gambling related words and phrases (Zack et al 2004). Basal dopamine transmission appears to be elevated in problem gamblers (Bergh et al 1997), and measurements taken during gameplay have suggested that dopamine transmission is likewise elevated in gamblers during winning streaks (Shinohara et al 1999). Despite these and other findings that have implicated dopamine in gambling and other reward-related behaviours, there is not a complete understanding of its contributions to gambling or the mechanisms by which it acts. Dopamine's role in modulating behaviour is complex; it is perhaps best understood as acting according to a "family of functions" whose effects vary according to task, cognitive demands, regional involvement and receptor subtype (see Floresco 2013 for review). Further exploration of dopaminergic involvement in behaviour (particularly gambling behaviour) could perhaps reveal therapeutic targets and reduce PG's public health impact.

Modeling gambling behaviour in laboratory animals

Laboratory-based tasks allow for the systematic examination of gambling behaviour, and animal models are particularly useful in the study of the neurobiological underpinnings of disorders of decision making. Several tasks have been designed to evaluate gambling-like behaviours in animals. The rodent Balloon Analogue Risk Task (rBART) measures individual risk sensitivity (Jentsch et al 2010). Animals are presented with two levers, one a reward lever and the other a "cash out" lever. A press of the reward lever increases the total amount of reward

that may ultimately be obtained, but each press is also associated with a chance of the loss of all reward accrued during that trial. The cash out lever allows the animal to collect the accrued reward. Experimental work with the rBART has demonstrated risk-aversion in rodents and a role for genetic influence on risky decision making (Jentsch et al 2010, Ashenhurst et al 2012), though no findings on dopamine's contributions to task performance have yet been published. The delay-discounting task (DD) enables animals to choose between a smaller-sooner and a larger-later reward, measuring the propensity to discount a reward as a function of the delay to its receipt. Preference for the smaller but more immediate reward is taken as a marker of impulsive choice, and psychostimulants (like those commonly prescribed for disorders of impulsivity such as ADHD) have been shown to increase choice of the delayed but greater reward (though some confounds exist, see Winstanley 2010). Higher rates of delay discounting have been shown to be related to poorer performance on gambling tasks (Monterosso et al 2001), and pathological gamblers show higher rates of discounting than healthy controls (Dixon et al 2003).

Similarly, probability-discounting tasks offer animals a choice between a smaller-certain reward and a larger-uncertain reward that becomes more or less likely over the course of a session. Pharmacological manipulations with this task have suggested that dopamine D_1 and D_2 receptor agonism increases risky choice, whereas D_3 agonism reduces risky choice (St Onge 2009). Clearly, dopamine's effects on decision making are not heterogeneous, but more research is needed to further clarify its role.

The aforementioned models, while useful in examining dimensions of decision making and outlining a role for dopamine in these processes, may not necessarily capture the specific type of decision making that is recruited in the context of gambling. For one, these tasks lack the ability to parse cost/benefit decision making across several concurrent schedules of reward and

punishment. Many forms of human gambling involve complex cost-benefit comparisons between options with varying amounts of risk and reward. The risk associated with a given decision is often ambiguous; the gambler usually does not know the explicit probability of receiving a reward for a given trial. The relative likelihood of reward must often be determined through trial and error, and even then the participant may only have a relative, but not complete, understanding of the contingencies of the task (Brand et al 2007). These tasks also fail to incorporate loss, an essential component of naturalistic gambling paradigms. Human gamblers must contend with this risk, which is distinct from the failure to win used as a substitute for true loss in many laboratory tasks (such as probability discounting). Models that encapsulate this element of loss, the implicit nature of risk in gambling and the complexity of possible choices represent a more ecologically valid approach to studying gambling behaviour.

Perhaps the most widely used task that fulfills these requirements is the Iowa Gambling Task (IGT), wherein human participants must choose between decks of cards with varying probabilities of monetary reward and punishment. The IGT provides a reliable measure of preference for risky (disadvantageous) over conservative (advantageous) options (Bechara et al 1994). As is the case in many real human gambling tasks, participants are able to choose between several options associated with different schedules of risk and reward. At the outset of the task, participants are instructed to choose between four decks of cards in order to maximize their winnings. Two of the decks (decks A and B) are associated with larger wins but also large losses, leading to a net loss over time. The remaining two decks (decks C and D) are associated with smaller wins but also smaller losses, and exclusive choice of these decks leads to a net gain over time. Subjects must learn to resist choosing the superficially tempting options (A and B) in order to succeed at the task, and work with the IGT has demonstrated impairment in a number of

clinical populations including pathological gamblers (Verdejo-Garcia et al 2007, Shurman et al 2005, Goudriaan et al 2005)). However, while the significance of these findings to the understanding of decision-making under conditions of risk and ambiguity should not be understated, the inherent limitations of human research (such as heterogeneous subject pools) prevents researchers from conducting many types of research into the specific pharmacology and neurobiology of these gambling-like behaviours.

The rodent gambling task (rGT)

To address these shortcomings, a rodent analogue of the IGT has recently been developed, known as the rat Gambling Task (rGT; Zeeb et al 2009). The rGT allows subjects to choose between four options, signaled by illumination of four response apertures, each with a unique probability of sucrose reward or "time-out" punishment. As in the IGT, the best strategy is to favor options associated with smaller gain but also smaller penalties, resulting in incremental maximization of sugar pellet profits. In contrast, a preference for the tempting "high-risk high-reward" outcomes is ultimately disadvantageous: although such options can yield greater rewards per trial, the disproportionately larger punishments result in considerably less benefit over time. Critically, this task incorporates loss, a central component of naturalistic gambling paradigms, through the use of punishing timeout periods. Given the limited length of each session, time is a resource animals are at risk of losing if their wager is unsuccessful. In essence, the disadvantageous options and their longer timeout periods require animals to balance the desire for larger rewards with the risk of the loss of future earning potential.

The rGT has allowed highly specific experimental manipulations and research with the task has begun to outline the neurobiology underlying this type of risky decision making. With respect to neurochemical regulation, work completed thus far has suggested that amphetamine

impairs decision-making on the rGT whereas the dopamine D_2 receptor antagonist eticlopride increased choice of the most profitable option (Zeeb et al 2009). However, administration of D_1-like or D_2-like agonists did not affect choice, which is somewhat surprising given the increase in risky behaviour observed in PD patients taking dopamine agonist therapy. Furthermore, administration of the selective dopamine reuptake inhibitor GBR 12909 did not affect decision making, although co-administration of this agent with the selective noradrenaline reuptake blocker did mimic the deleterious effects of amphetamine (Baarendse et al 2012). Furthermore, while both D_1 and D_2-family antagonists can attenuate impulsive responses caused by amphetamine, neither of these compounds could attenuate amphetamine-induced impairments in choice (Zeeb et al 2013). In sum, choice behaviour on the rGT does not seem to be predominantly driven by the dopamine system.

Cue-mediated decision making

While research with the rGT thus far has provided valuable insight into gambling-like behaviour, there is still much room for exploration and refinement. An area of interest for further investigation is the role of salient cues in guiding decision-making. Highly salient win-associated cues are a significant component of human gambling, and may play an important role in dopamine's ability to modulate gambling behaviour. Cues have been shown to increase the release of dopamine (Schultz et al 1997), and losses that bear visual similarities to wins can increase the desire to gamble, especially in problematic gamblers (Clark et al 2010). Animals' propensity to orient to a reward-related cue over the reward itself is correlated with the expression of D_1 in the nucleus accumbens (Flagel et al, 2009), a structure that has been repeatedly shown to be critical in the development and maintenance of compulsive and addictive behaviours. Findings such as these suggest that dopamine's role in mediating decision making

(and by extension, gambling behaviour) may be inexorably tied to salient cues. Cues are additionally thought to play a role in individual sensitivity to addiction and the propensity to relapse (Everitt et al 2000, Kruzich et al 2001). The development of a model that incorporates both salient cues and decision-making would allow the concurrent exploration of these two significant elements of maladaptive behaviour.

The theory of incentive salience proposes a possible role for dopamine in cue-mediated behaviour. The theory suggests that "reward is a composite construct that contains multiple component types: wanting, learning, and liking. Dopamine mediates a 'wanting' component, by mediating the dynamic attribution of incentive salience to reward-related stimuli, causing them and their associated reward to become motivationally 'wanted' (Berridge 2007)". Reward-related cues themselves will therefore become "wanted" or motivationally salient and capable of driving behaviour to a greater extent than reward alone could (Heinz et al 2004). This characterization of dopamine could explain why changes in dopamine levels seem to exert significant effects on reward-related behaviour (eg dopamine agonists and the development of pathological gambling in Parkinson's patients), especially in the context of salient, reward-related cues.

However, these theories are largely derived from associative learning studies in which animals come to pair a salient stimulus with the delivery of reward. While these paradigms provide empirical evidence for the power of cues to invoke appetitive behaviours, they do not examine the relationship between dopamine function and decision making. The development and maintenance of addictive and compulsive behaviours are more complex than the simple pairing of a cue and reward. A task incorporating salient cues into the decision-making process could reveal much more about the pharmacology of this behaviour than the simple Pavlovian tasks currently in use.

Cue-mediated decision making and dopaminergic involvement is a fallow area for research. Many existent animal tasks incorporate intentional and unintentional cues. One discussion of delay discounting literature proposed that the divergent and seemingly contradictory effects of dopaminergic compounds on choice behaviour may be due to the presence or absence of cues in the task (Zeeb 2010). The authors argued that cues presented during the delay effectively "bridged the gap" on larger but delayed rewards, acquiring some of the appetitive properties of the reward and increasing its subjective value. Indeed, when the delay was explicitly cued, prefrontal infusions of D_1 and D_2 antagonists increased risky choice, an effect that was not observed when the delay was uncued. Clearly, cues can have tremendous influence on goal-directed behaviour and are a rich subject for investigation. However, to our knowledge, there are no animal tasks that pair highly salient cues with complex decision making. This is a potentially rich subject for exploration; human gambling often demands decision-making in the context of salient cues, and attentional bias towards these cues may play a critical role in the transition from recreational to problem gambling (van Holst et al. 2012; Grant and et al2014). Understanding the influence of these cues on decision making could therefore provide valuable insight into pathological gambling and other cognitive biases.

In order to explore the role of cues in shaping choice behaviour, we disproportionally cued wins on the rGT's disadvantageous options to see if these cues can shift animals' decision-making preferences. The pairing of salient cues to disadvantageously risky options is similar to human gambling paradigms in which large, often risky wins are more saliently cued than small wins or losses. Following training on the task, animals underwent a number of pharmacological challenges in order to determine whether the addition of these reward-related cues had altered the effect of dopaminergic drugs on choice behaviour. This model will give us more insight into cue-

mediated decision making and its neurobiological underpinnings.

Methods

Subjects

Subjects were 16 male Long-Evans rats (Charles Rivers Laboratories, St. Constant, Quebec, Canada) weighing 250-275g at the time of arrival at the animal facility. Animals were food restricted to 85% of free feeding weight and maintained on a diet of 14g of standard rat chow per day. Water was available ad libitum in home cages. Animals were pair-housed and maintained in a climate-controlled colony room on a 12-hour reverse light cycle (lights off at 0800). All experimental work was approved by the University of British Columbia's Animal Care Committee and husbandry was performed in accordance with the standards set forth by the Canadian Council of Animal Care.

Behavioural apparatus

Testing took place in 16 standard Med Associates 5-hole operant chambers housed in ventilated sound-attenuating cabinets (Med Associates Inc, Vermont, USA). Each chamber featured a food magazine outfitted with both a stimulus light and an infrared beam for detecting nose-poke inputs. 45mg sucrose pellets (Bio-Serv, New Jersey, USA) could be delivered to the magazine from an external food hopper. A house light was positioned above the magazine. An array of five response apertures was located on the opposite wall, each equipped with stimulus lights and infrared beams for detecting input. The operant chambers ran on MedPC programs authored by CAW controlled by an IBM-compatible computer.

Behavioural testing

Operant training

Animals were initially habituated to the operant chambers over the course of two 30 minute exposures during which sucrose pellets were placed in each of the apertures and animals

were allowed to explore the apparatus. Animals then trained on a variant of the 5-CSRTT in which one of the five nose-poke apertures was illuminated for 10 seconds and a nose-poke response was rewarded with a single sucrose pellet delivered to the food magazine. The aperture in which the stimulus light was illuminated varied across trials. Each session consisted of 100 trials and lasted 30 minutes. Animals were trained on this task until responding reached 80% accuracy and fewer than 20% omissions. Once this training was complete, rats trained on a forced-choice variant of the rGT. This training procedure was designed so that animals were forced to respond an equal number of times to each aperture that would be utilized in the rGT (from left to right: 1, 2, 4 and 5) in order to ensure equal exposure to the contingencies associated with each hole and minimize any potential primacy effects. The contingencies on this task were the same as those used in the full versions of the rGT (detailed below).

The Flash Preference task

Following training, animals were tested on the Flash Preference task (FPt), a procedure designed to measure the affective qualities of flashing stimulus lights. This task was designed with the intention of determining whether cue lights flashing at different frequencies were appetitive or aversive before using them as appetitive stimuli in the cued rGT. Each session of the FPt lasted 30 minutes. There was no limit to the number of trials an animal could initiate. Like the rGT, a trial began with the illumination of the tray light. A nose-poke response turned the tray light off and began a five-second inter-trial interval (ITI). At the end of the ITI, two apertures on the opposite wall flashed and a nose-poke in either of the illuminated apertures was rewarded with the delivery of a sugar pellet to the food tray. Two distinct frequencies were displayed during each trial. The cue lights in the illuminated apertures flashed at a rate in the range of one to five hertz, and the location and frequency of the two lights varied across trials.

There was no optimal strategy on the FPt; a response in either of the illuminated apertures would always result in the delivery of a single sugar pellet. If the animal failed to make a response in either of the apertures within 10 seconds, the trial was scored as an omission, the aperture lights extinguished and the tray light turned on once again to allow the animal to initiate a new trial.

The rGT

A task schematic is provided as figure 1. Each trial began with the illumination of the tray light. A nose-poke response in the tray turned the tray light off and began a five-second ITI during which all lights were extinguished and the animal had to refrain from responding to any of the apertures. Following the ITI, cue lights in the response apertures one, two, four and five were illuminated by a solid cue light on each trial. A nosepoke response at an illuminated aperture was then either rewarded or punished, according to the unique reinforcement schedule associated with that aperture. If the response was rewarded, the aperture light would be extinguished, the tray light would be illuminated and the appropriate number of sucrose pellets would be distributed. The animal's response in the tray extinguished the tray light and initiated a new trial. If the response was punished, a time-out period commenced during which the selected aperture flashed at a rate of 0.5 hertz for the duration of the punishment and the animal was unable to make a response. At the end of the timeout period, the aperture light turned off, the tray light turned on, and the animal was able to begin a new trial by responding to the tray. If the animal responded in any aperture during the ITI, the trial was scored as a premature response, and the house light turned on to mark a 5 second time-out period during which the animal would be unable to register a response. At the end of the time-out period, the house light turned off, the tray light turned on, and the animal could initiate a new trial.

The different schedules of reward and punishment associated with each aperture resulted

in unequal return across a session. Option one, hereafter referred to as P1, was associated with a 90% probability of a return of one sucrose pellet and a 10% probability of a five-second timeout period. Option two, or P2, was associated with an 80% probability of a return of two sucrose pellets and a 20% probability of a 10 second timeout period. Option three (P3) was associated with a 50% probability of a return of three sucrose pellets and a 50% probability of a 30 second timeout period. Option four (P4) was associated with a 40% probability of a return of four sucrose pellets and a 60% probability of a 40 second timeout period. The optimal strategy was exclusive choice of P2 over the course of the 30 minute session, as the expected return for this pattern of selection would be approximately 411 sucrose pellets. Likewise, exclusive choice of P1 would return approximately 295 sucrose pellets, P3 approximately 135 sucrose pellets and P4 approximately 99 sucrose pellets. Although the return on individual winning trials was higher for options P3 and P4, the higher frequency and longer duration punishments associated with these options made their selection disadvantageous over time. The position of each option was counterbalanced across animals to mitigate any potential side biases. Version A (n=8) was arranged P1, P4, P2, P3 from left to right, and version B (n=8) was arranged P4, P1, P3, P2. A total of 16 animals were tested on this version of the task, while the remaining 16 were tested on the cued rGT.

The cued rGT

The structure of the cued rGT was identical to that of the traditional rGT, save the introduction of salient cues to winning trials. On the cued rGT, a loss on any option was identical to a loss on that same option on the traditional rGT. However, while a win on the rGT was marked by the allocation of sucrose pellets and the solid illumination of the tray light, a win on the cued rGT was additionally marked by a combination of tones and flashing lights, varying in

complexity across options. Like a human gambling paradigm, the magnitude of win-associated cues became disproportionately larger as win size increased. A rewarded selection of P1 or P2 was marked by less salient cues, and a win on P3 and P4 was marked by more salient cues.

Each win-associated cue lasted for a period of two seconds. On a rewarded P1 trial, the corresponding aperture flashed at one hertz and the tray light was solidly illuminated. A single tone played concurrently with the flashing cue light. Likewise, a rewarded P2 trial was marked by the cue light in the corresponding aperture flashing at a rate of one hertz, and the tray light was again solidly illuminated. A win on P2 was also marked by a tone sequence composed of two distinct tones lasting one second each.

A win on P3 or P4 had more complex cues. On a winning trial, the winning aperture flashed for the first second and was then followed by a one-second sequence of cue lights flashing at five hertz. Lights were illuminated both together and individually. Winning P3 trials were associated with one of two patterns of three flashing lights; each pattern was composed of the P3 cue light and the two most proximate apertures. Winning P4 trials were associated with one of four sequences of flashing lights; each sequence was made up of a unique combination all five apertures lights flashing. Wins were also marked by tone sequences. P3 wins featured one of two tone sequences, each a ten-tone sequence lasting two seconds and composed of three unique tones. P4 wins featured one of four distinct tone patterns. Each P4 tone pattern was a ten-tone sequence composed of six unique tones.

Behavioural measurements

A number of behavioural measurements were taken during the task. Choice of each individual option was calculated as [(all choices of a given option)/(total trials completed)]*100. Calculating choice preference as a percentage of all choices rather than as a raw count of total

choices controlled for differences in total trials executed across sessions and between animals. A measure termed the "score variable" was developed to communicate to what extent an animal's choice was optimal. As is often used to represent data obtained from the IGT (Bechara et al 1999), the score variable was defined as the difference between choice of the advantageous options and the disadvantageous options, and was calculated according to the following formula: {[(choice of P1)+(choice of P2)]-[(choice of P3)+(choice of P4)]}. A higher score indicated more advantageous choice strategy, whereas a lower score indicated a more disadvantageous choice strategy.

As previously described, any response made during the ITI was scored as a premature response, and these were calculated as [(total premature responses)/(total trials initiated)]*100. As with choice preference, this formula yielded a percentage score. Latency to choose an option was calculated as the time between the end of the ITI and a response in any of the apertures. Latency to collect reward was calculated as the time between reward delivery and the animal's subsequent nose-poke response in the tray. Both choice and collection latency were averaged across session for each option. Behavioural testing continued until statistically stable performance was established, defined asno main effect of session or choice x session interaction term when analyzing data from 3 consecutive days).

Drugs

Pharmacological manipulations began once animals had achieved stable baseline responding, defined as a non-significant effect of session and choice x session interaction on a repeated measures ANOVA across the previous three sessions. All drugs were prepared fresh daily, and the order in which doses were administered was determined by a Latin-Square design. Each drug was administered in three-day cycles; the first day was a baseline session, the second

a drug administration day, and the third a rest day in which animals were not tested and remained in the home cage. Drugs were administered ten minutes before the start of behavioural testing. To prevent any potential carryover effects, animals were given a washout period between drugs of at least one week. During this period, they were tested on the task.

Drug doses are provided in table 1. All doses were calculated as the salt. d-amphetamine sulfate, quinpirole, eticlopride and PD-168077 were purchased from Sigma-Aldrich (Oakville, Canada). A-381393 was a gift from Dr. Anton Pekcec of Boehringer Ingelheim (Ingelheim, Germany). All drugs were delivered via intraperitoneal (IP) administration. Drugs were dissolved at a volume of 1ml/kg in 0.9% sterile saline, with the exception of A-381393 which was dissolved in a solution of 40% 0.1M hydrochloric acid. The order of administration was as follows: d-amphetamine, quinpirole, eticlopride, PD-168077, A-381393.

Data analysis

All data analysis was performed with SPSS for Mac (Version 22.0.0; IBM). Percentage variables were arcsine transformed to minimize artificial ceiling effects. Significance was set at the $p<0.05$ level for all data analysis. Repeated-measures ANOVAs were used to analyze data, with choice (four levels, P1-P4), session and drug dose (four levels, vehicle + three doses of drug) as within-subjects factors, and group as a between-subjects factor. One animal in the uncued group was excluded from all analyses due to unresolved behavioral instability.

Results

Flash Preference

Animals showed a preference for cue lights flashing at higher frequencies (Figure 2; Choice: $F_{4,56}$=12.714, p<.001). Choice of the three, four and five hertz options were significantly higher than choice of the one and two hertz options (Table 2). However, there was no group by choice effect when the animals were grouped according to the version of the rGT they would later be tested on (group x choice: $F_{4,56}$=.575, NS), suggesting there were no preexisting differences between groups in their preference for or aversion to flashing lights.

Baseline behavior

Both groups reached behavioral stability at the same time point (Sessions 35-37; Session x Choice: $F_{6,78}$=1.415, NS; Session x Choice x Cue: $F_{6,78}$= .558, NS). Animals performing the cued rGT demonstrated a significantly more disadvantageous choice preference as compared to animals performing the uncued rGT, as measured by the score variable (Figure 3;Group: $F_{1,13}$= 5.694, p=.033). Average choice score for the cued task (mean: -7.28, +/-16.21), indicated a slight preference for the disadvantageous options, whereas the average choice score for animals on the uncued task (mean: 45.52, +/-14.68) indicated a stronger preference for the advantageous options. When individual choice options were considered, behaviour on the cued rGT was highly variable. On average, rats performing the cued task chose the best option less frequently (Figure 4; P2: $F_{1,13}$=5.129, p = 0.041). However, this was not sufficient to dissociate performance of the two groups when data from all choices were compared together (Group: $F_{1,13}$=.155, NS; Group x Choice: $F_{3,42}$=2.301, NS).

Choice latency did not differ between the cued and uncued groups (Figure 5; Group: $F_{1,13}$=.779, NS), nor did collection latency (Figure 5; Group: $F_{1,13}$=2.928, NS). There were no

differences in premature responses (Figure 6; Group: $F_{1,13}$=.535, NS) nor omissions (Group: $F_{1,13}$=.711, NS) between groups. Animals performed similar number of trials across both tasks (Group: $F_{1,13}$= 3.552, NS).

Amphetamine

In keeping with previous reports, amphetamine increased choice of P1 across all animals, regardless of which task they were performing (Figure 7, Figure 8; Dose x Choice: $F_{9,117}$=2.776, p=.006; Dose x Choice x Group: $F_{9,117}$=.663, NS). The highest dose of amphetamine also increased choice latency across both groups (Figure 9; Dose: $F_{3,39}$=6.504, p=.001; Dose x Group: $F_{3,39}$=.456, NS; saline v 1.5 mg/kg: t(13)=2.903, p=.012) and increased the latency to collect reward (Figure 9; $F_{3,39}$=4.879, p=.006; Dose x Group: $F_{3,39}$=.1.168, NS), indicating some motor slowing at this dose. Surprisingly, amphetamine did not induce a robust increase in premature responding in either cohort, and appeared to reduce this measure of motor impulsivity at the highest dose tested (Figure 10; Dose: $F_{3,39}$=6.878, p=.001; Dose x Group: $F_{3,39}$=.547, NS). However, no single dose produced a significant difference in this variable as compared to saline treatment, making these data hard to interpret.

Amphetamine decreased trials at higher doses in both groups (Dose: $F_{3,39}$= 2.992, p=.042; Dose x Group: $F_{3,39}$= 1.321, NS). There was no effect on omissions (Dose: $F_{3,39}$= 2.854, NS; Dose x Group: $F_{3,39}$= .136, NS).

Eticlopride

In contrast to previous reports (Zeeb et al, 2009, 2013), the D_2 antagonist eticlopride did not improve performance by increasing choice of the best option in the uncued paradigm (Figure 12), and a similar null effect was observed in the cued version of the task (Figure 11) (Dose: $F_{3,33}$= 1.642, NS; Dose x Choice: $F_{9,99}$= .758, NS; Dose x Choice x Group: $F_{9,99}$=1.094, NS).

Eticlopride increased the latency to make a choice (Figure 13; Dose: $F_{3,33}$= 6.621, p=.001; Dose x Group: $F_{3,33}$= 1.641, NS), and decreased premature responses (Figure 14; Dose: $F_{3,33}$=14.098, p<.001; Dose x Group: F 3,33=1.406, NS) in both the cued and the uncued groups. There were no effects on any other behavioral measure.

Quinpirole

In contrast to previous reports, the $D_{2/3}$ receptor agonist quinpirole altered choice of both P1 and P2 (Dose x Choice: $F_{9,117}$=2.686, p=.007; Dose x Choice x Group: $F_{9,117}$=1.952, p=.05; P1: F 3,39= 3.431, p=.026; P2: F 3,39= 4.948, p=.005). Analysing data from each group separately confirmed that only behaviour of the cued group was affected by the drug, increasing choice of P1 and P4, and decreasing choice of P2 (Figure 16: Uncued group: Dose x Choice: $F_{9,54}$= 1.067, NS; Figure 15: Cued group: Dose x Choice: $F_{9,63}$= 4.128, p<.001; Dose: P1: $F_{3,21}$= 3.390, p=.007; P2: $F_{3,21}$=10.784, p<.001; -P4: $F_{3,21}$=2.932, p=.05).

Quinpirole decreased premature responses (Figure 18; Dose: $F_{3,39}$= 22.722, p<.001; Dose x Group: $F_{3,39}$=.391, NS) and increased choice latency (Figure 17; Dose: $F_{3,39}$= 31.474, p<.001; Dose x Group: $F_{3,39}$= 1.156, NS) in both groups. No other behavioral measure was significantly affected.

A-381393

A-381393, a selective D_4 receptor antagonist, did not significantly affect choice in either group (Figure 19, Figure 20; Dose: $F_{3,39}$= .279, NS; Dose x Choice: $F_{9,117}$= .209, NS) or any other behavioural measures (Figure 21; Figure 22).

PD-168077

The D_4 receptor agonist PD-168077 did not affect choice behaviour in either the cued or uncued groups (Figure 23, Figure 24; Dose: $F_{3,39} = .279$, NS; Dose x Choice: $F_{9,117} = .209$, NS). All other behavioural measures were likewise unaffected (Figure 25; Figure 26).

Table 1: Drug doses

Drug name	Drug type	Doses	(mg/kg)		
Amphetamine	Non-selective dopamine agonist	0.3	1.0	1.5	0
Quinpirole	Dopamine D2 family agonist	0.0125	0.0375	0.125	0
Eticlopride	Dopamine D2 family antagonist	0.01	0.03	0.06	0
PD-168077	Dopamine D4 agonist	0.5	1.0	5.0	0
A-391383	Dopamine D4 antagonist	0.5	1.0	5	0

Table 1: Drug doses. All doses were calculated as the salt. All drugs were delivered via intraperitoneal (IP) administration. Drugs were dissolved at a volume of 1ml/kg in 0.9% sterile saline, with the exception of A-381393 which was dissolved in a solution of 40% hydrochloric acid. The order of administration was as follows: d-amphetamine, quinpirole, eticlopride, PD-168077, A-381393. All drug effects were compared to the effects of an injection of the vehicle used for each drug (indicated by a dose of 0 mg/kg on the table).

Table 2: Flash Preference test

Light Frequency (Hertz)	T value	Significance
1 vs 2	t(15)= 2.474	p= .026
1 vs 3	t(15)= 4.238	p= .001
1 vs 4	t(15)= 4.732	p<.001
1 vs 5	t(15)= 5.268	p<.001
2 vs 3	t(15)= 3.532	p= .003
2 vs 4	t(15)= 3.579	p= .003
2 vs 5	t(15)= 3.500	p= .003
3 vs 4	t(15)= .497	p= .626
3 vs 5	t(15)= 1.215	p= .243
4 vs 5	t(15)= .867	p= .400

Table 2: Flash Preference test. Animals showed a preference for cue lights flashing at higher frequencies (Figure 2; Choice: $F_{4,56}$=12.714, p<.001). Choice of the three, four and five hertz options were significantly higher than choice of the one and two hertz options.

Figure 1: Schematic of the rodent gambling task

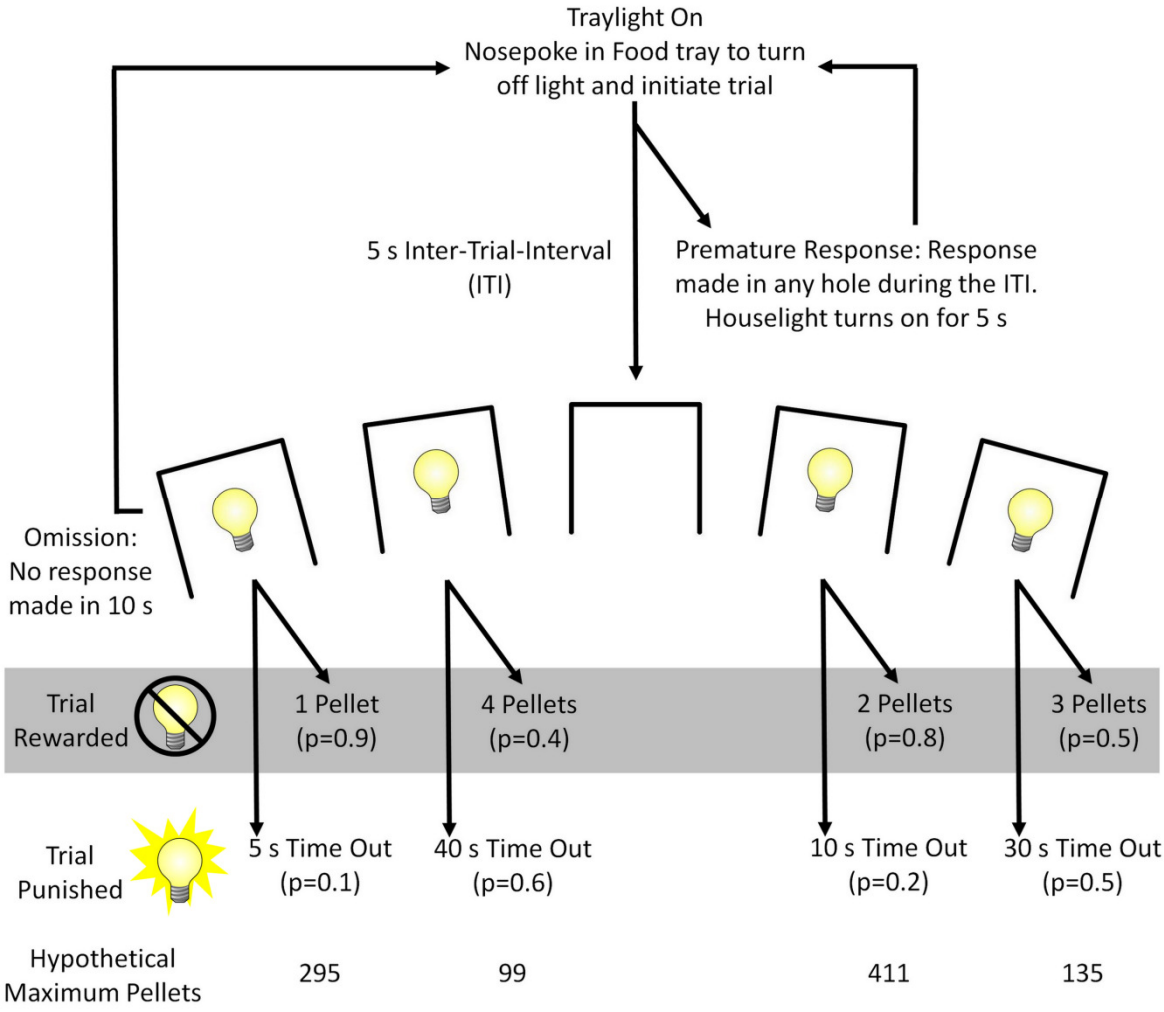

Figure 1: Schematic of the rodent gambling task. Each trial began with the illumination of the tray light. A nose-poke response in the tray turned the tray light off and began a five-second ITI. Following the ITI, cue lights in the response apertures were illuminated. A nosepoke response at an illuminated aperture was then either rewarded or punished, according to the unique reinforcement schedule associated with that aperture. If the response was rewarded, the aperture light would be extinguished, the tray light would be illuminated and the appropriate number of sucrose pellets would be distributed. If the response was punished, a time-out period commenced during animal was unable to make a response. At the end of the timeout period, the aperture light turned off, the tray light turned on, and the animal was able to begin a new trial by responding to the tray. If the animal responded in any aperture during the ITI, the trial was scored as a premature response, and the house light turned on to mark a 5 second time-out period during which the animal would be unable to register a response.

Figure 2: Flash Preference test

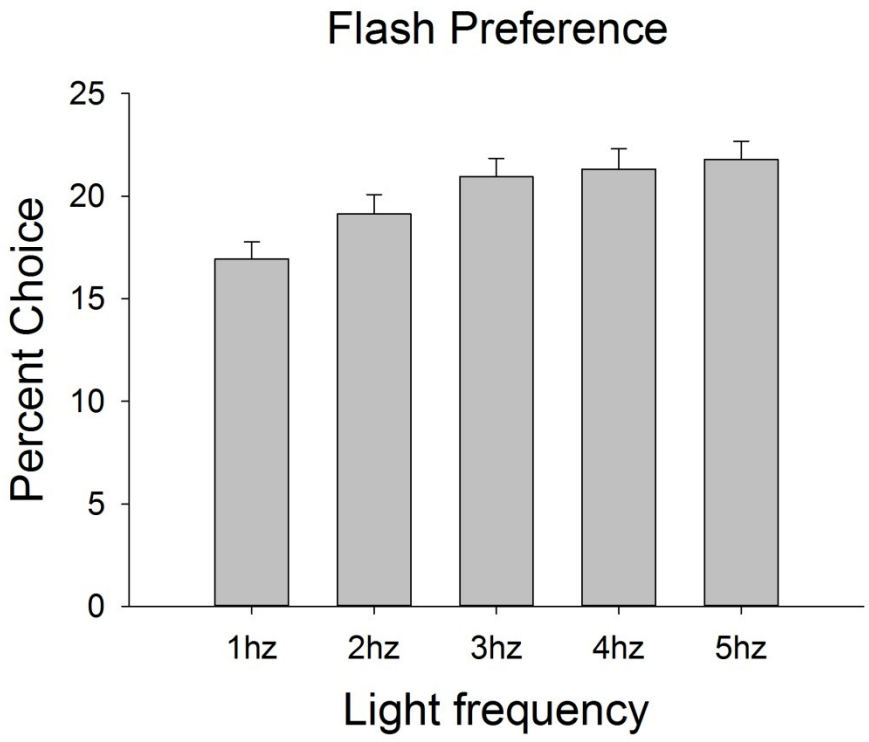

Figure 2: Flash Preference test. Animals showed a preference for cue lights flashing at higher frequencies (Choice: $F_{4,56}$=12.714, p<.001). Choice of the three, four and five hertz options were significantly higher than choice of the one and two hertz options (see Table 2). Data are shown as mean +/- SEM.

Figure 3: Baseline score variable by group

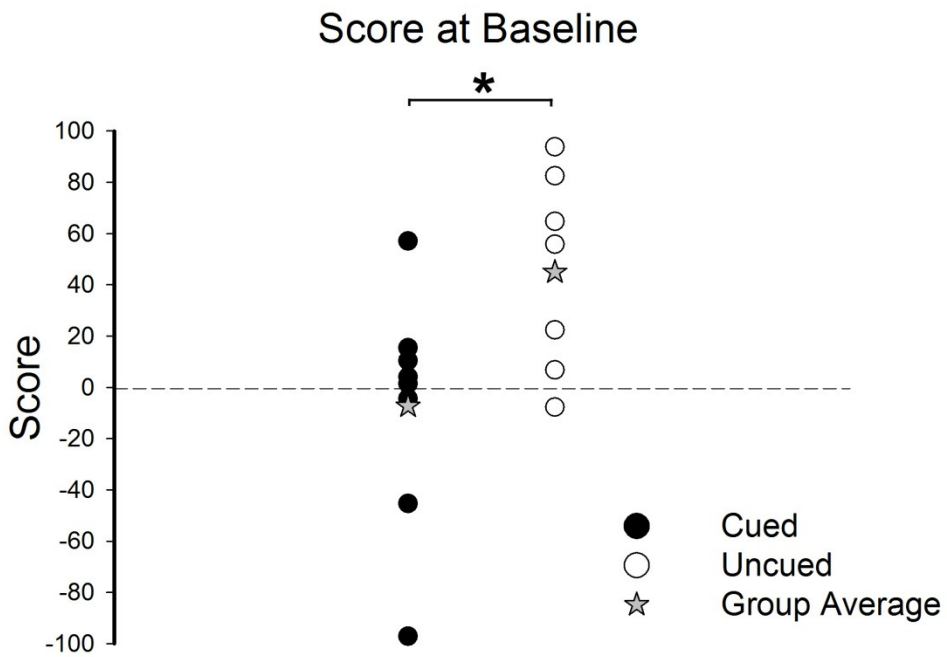

Figure 3: Baseline score variable by group. Animals performing the cued rGT demonstrated a significantly more disadvantageous choice preference as compared to animals performing the uncued rGT, as measured by the score variable (Group: $F_{1,13}= 5.694$, p=.033). Average choice score for the cued task (Mean: -7.28, +/-16.21), indicated a slight preference for the disadvantageous options, whereas the average choice score for animals on the uncued task (Mean: 45.52, +/-14.68) indicated a stronger preference for the advantageous options.

Figure 4: Baseline choice preference by group

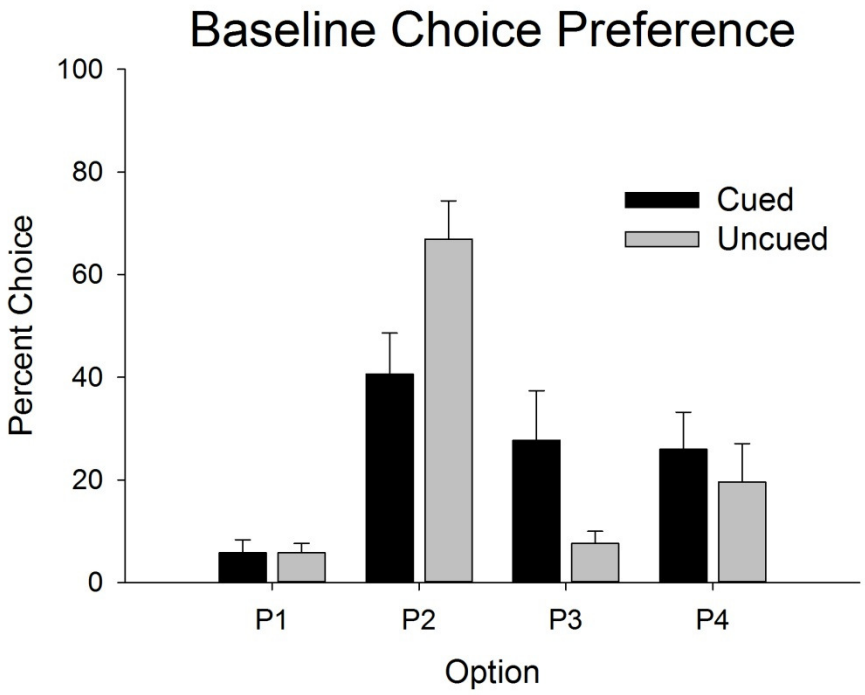

Figure 4: Baseline choice preference by group. When individual choice options were considered, behaviour on the cued rGT was highly variable. On average, rats performing the cued task chose the best option less frequently (P2: $F_{1,13}$=5.129, p = 0.041). However, this was not sufficient to dissociate performance of the two groups when data from all choices were compared together (Group: $F_{1,13}$=.155, NS; Group x Choice: $F_{3,42}$=2.301, NS). Data are shown as mean +/- SEM.

Figure 5: Baseline latencies

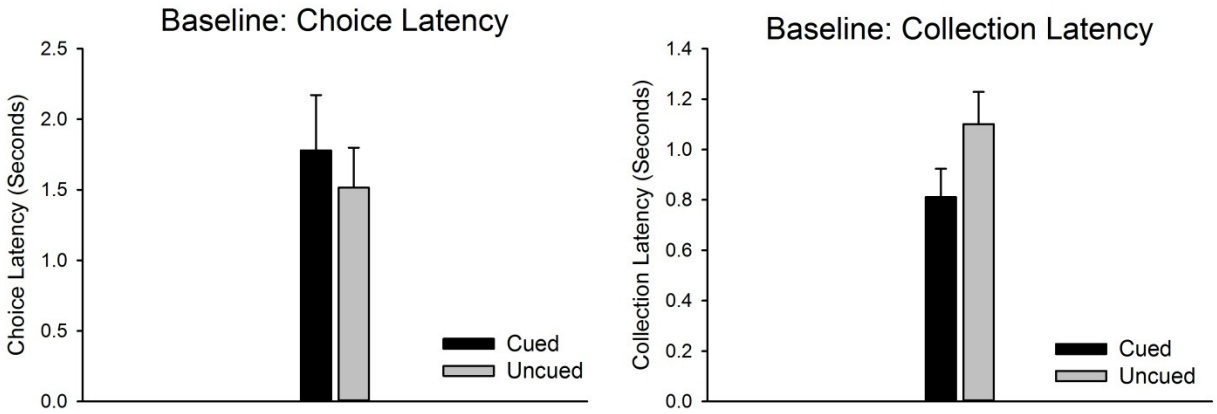

Figure 5: Baseline latencies. Choice latency did not differ between the cued and uncued groups (Group: $F_{1,13}$=.779, NS), nor did collection latency (Group: $F_{1,13}$=2.928, NS). Data are shown as mean +/- SEM.

Figure 6: Baseline premature responding

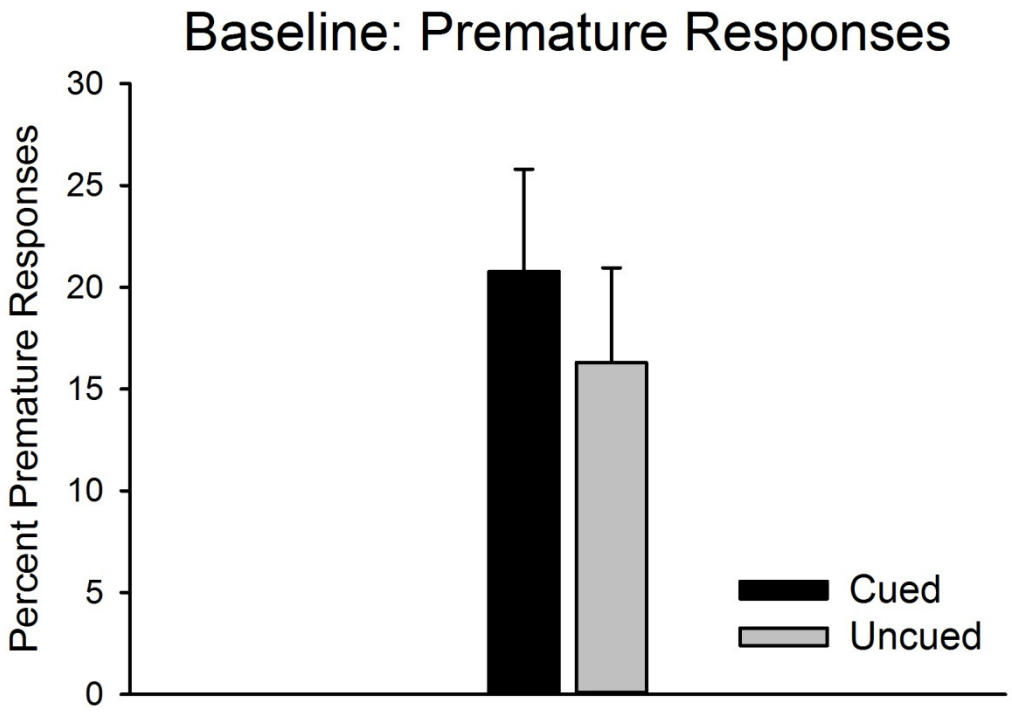

Figure 6: Baseline premature responding. There were no differences in premature responses (Figure 5; Group: $F_{1,13}$=.535, NS) between groups at baseline. Data are shown as mean +/- SEM.

Figure 7: Effects of amphetamine on choice preference in the cued task

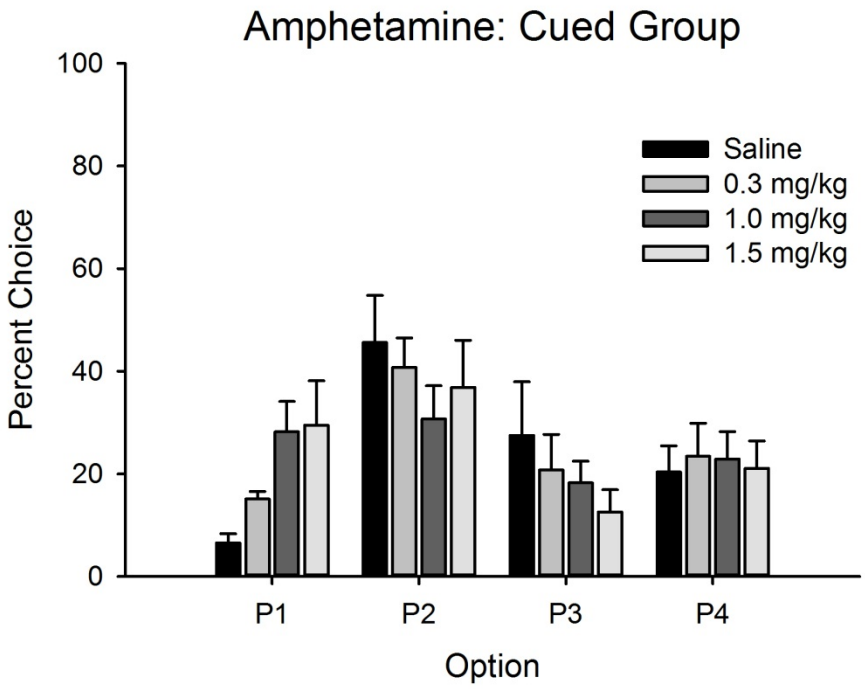

Figure 7: Effects of amphetamine on choice preference in the cued task. In keeping with previous reports, amphetamine increased choice of P1 across all animals, regardless of which task they were performing (Dose x Choice: $F_{9,117}$=2.776, p=.006; Dose x Choice x Group: $F_{9,117}$=.663, NS). Data are shown as mean +/- SEM.

Figure 8: Effects of amphetamine on choice preference in the uncued task.

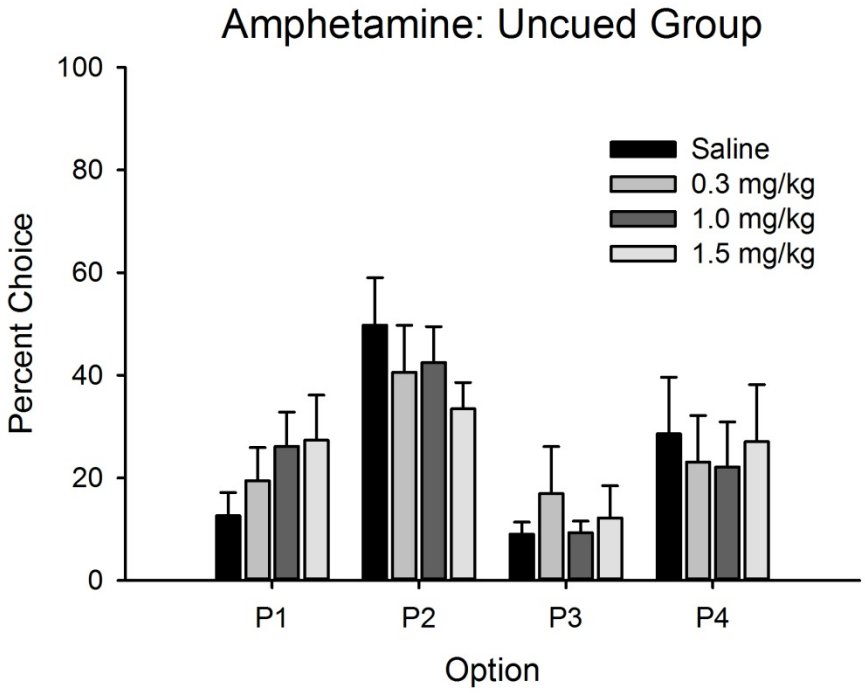

Figure 8: Effects of amphetamine on choice preference in the uncued task. In keeping with previous reports, amphetamine increased choice of P1 across all animals, regardless of which task they were performing (Dose x Choice: $F_{9,117}=2.776$, $p=.006$; Dose x Choice x Group: $F_{9,117}=.663$, NS). Data are shown as mean +/- SEM.

Figure 9: Effects of amphetamine on latencies.

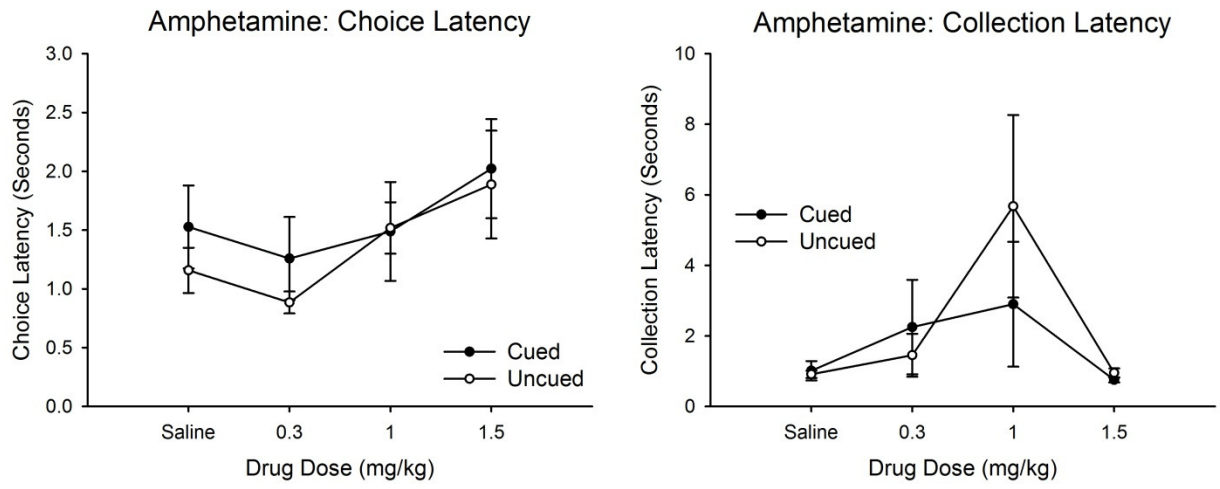

Figure 9: Effects of amphetamine on latencies. The highest dose of amphetamine also increased choice latency across both groups (Dose: $F_{3,39}$=6.504, p=.001; Dose x Group: $F_{3,39}$=.456, NS; saline v 1.5 mg/kg: t(13)=2.903, p=.012) and increased the latency to collect reward ($F_{3,39}$=4.879, p=.006; Dose x Group: $F_{3,39}$=.1.168, NS), indicating some motor slowing at this dose. Data are shown as mean +/- SEM.

Figure 10: Effects of amphetamine on premature responding.

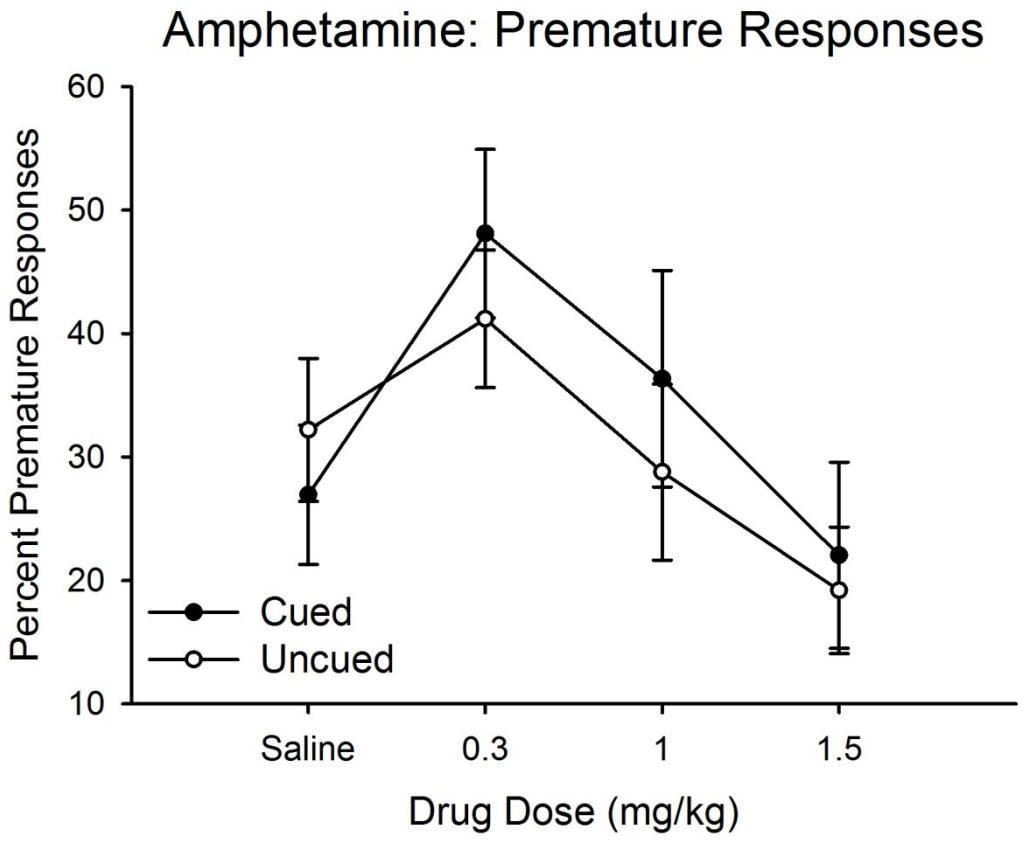

Figure 10: Effects of amphetamine on premature responding. Surprisingly, amphetamine did not induce a robust increase in premature responding in either cohort, and appeared to reduce this measure of motor impulsivity at the highest dose tested (Dose: $F_{3,39}$=6.878, p=.001; Dose x Group: $F_{3,39}$=.547, NS). However, no single dose produced a significant difference in this variable as compared to saline treatment, making these data hard to interpret. Data are shown as mean +/- SEM.

Figure 11: Effects of eticlopride on choice preference in the cued task.

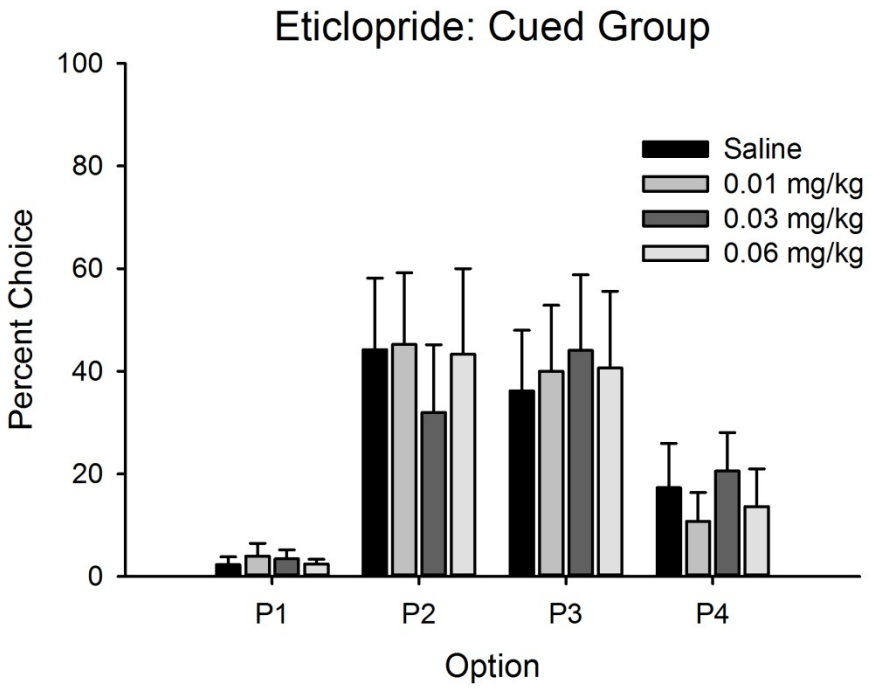

Figure 11: Effects of eticlopride on choice preference in the cued task. The D_2 antagonist eticlopride did not change performance on the cued version of the task (Dose: $F_{3,33}$= 1.642, NS; Dose x Choice: $F_{9,99}$= .758, NS; Dose x Choice x Group: $F_{9,99}$=1.094, NS). Data are shown as mean +/- SEM.

Figure 12: Effects of eticlopride on choice behaviour in the uncued task.

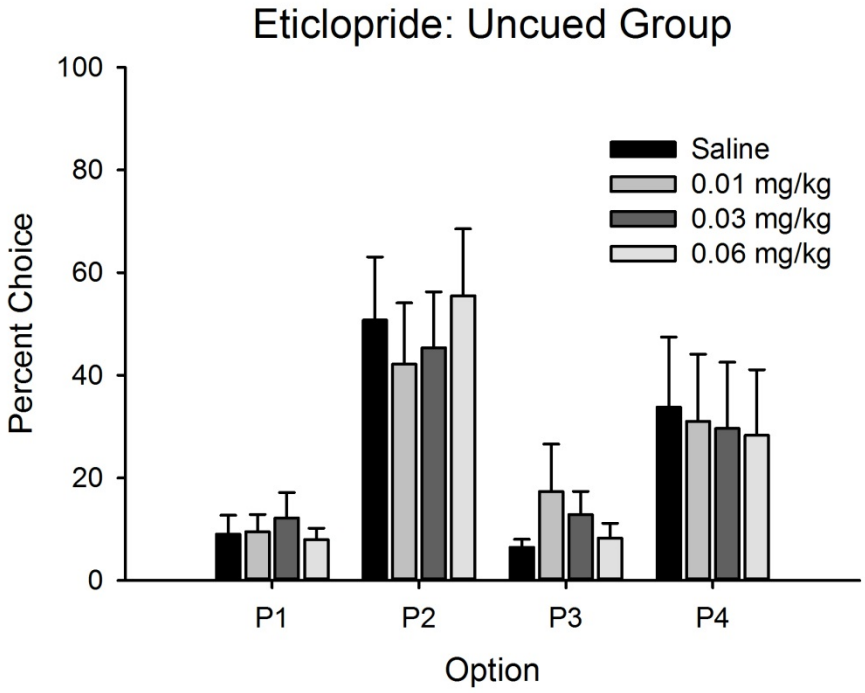

Figure 12: Effects of eticlopride on choice behaviour in the uncued task. In contrast to previous reports (Zeeb et al, 2009, 2012), the D_2 antagonist eticlopride did not improve performance by increasing choice of the best option in the uncued paradigm (Dose: $F_{3,33}$= 1.642, NS; Dose x Choice: $F_{9,99}$= .758, NS; Dose x Choice x Group: $F_{9,99}$=1.094, NS). Data are shown as mean +/- SEM.

Figure 13: Effects of eticlopride on latencies.

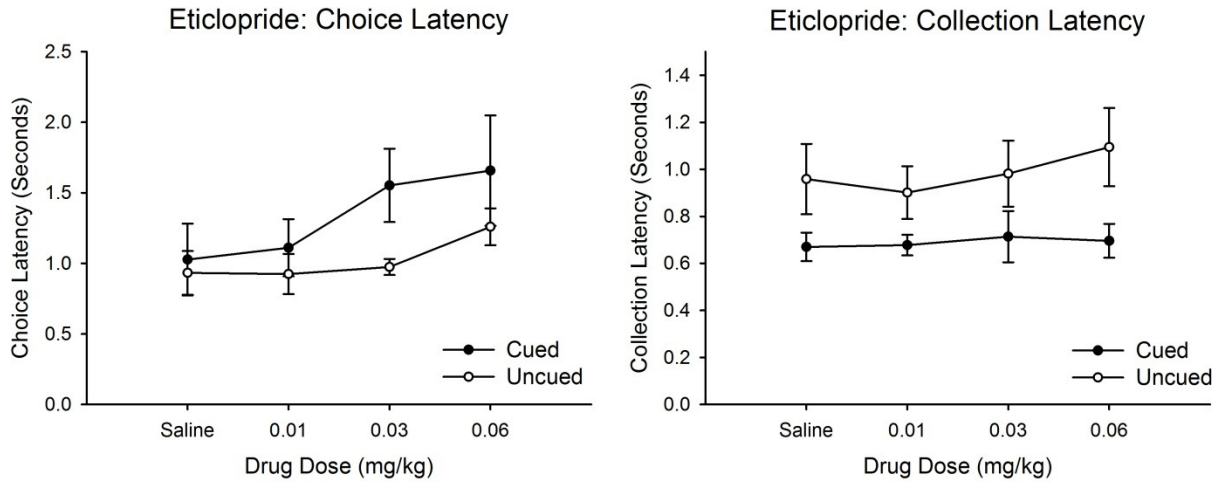

Figure 13: Effects of eticlopride on latencies. Eticlopride increased the latency to make a choice (Dose: $F_{3,33}$= 6.621, p=.001; Dose x Group: $F_{3,33}$= 1.641, NS). There was no effect on collection latency. Data are shown as mean +/- SEM.

Figure 14: Effects of eticlopride on premature responding.

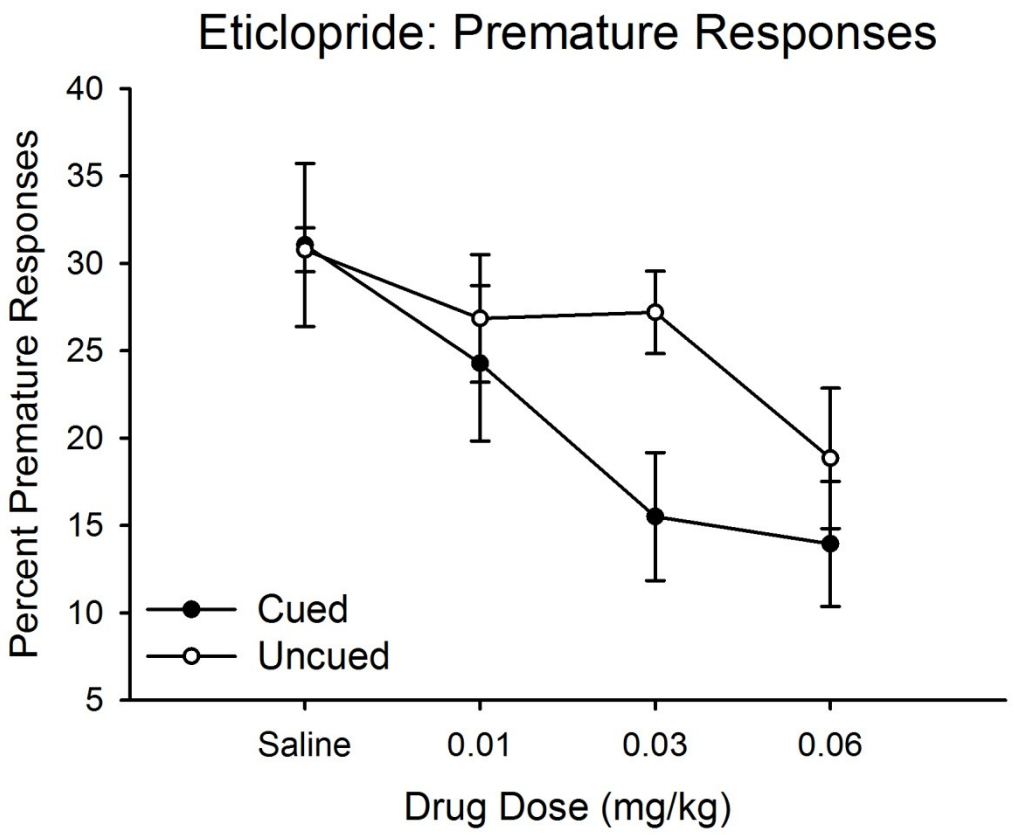

Figure 14: Effects of eticlopride on premature responding. Eticlopride decreased premature responses (Dose: $F_{3,33}$=14.098, p<.001; Dose x Group: F 3,33=1.406, NS) in both the cued and the uncued groups. Data are shown as mean +/- SEM.

Figure 15: Effects of quinpirole on choice behavior in the cued task.

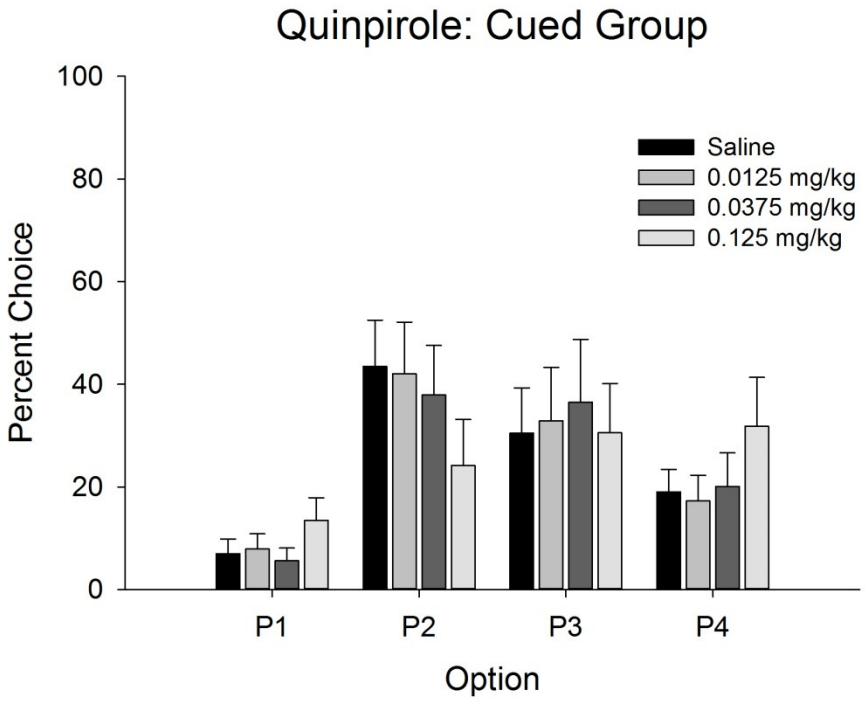

Figure 15: Effects of quinpirole on choice behavior in the cued task. Only behaviour in the cued group was affected by quinpirole, as it increased choice of P1 and P4, and decreased choice of P2 (Cued group: Dose x Choice: $F_{9,63}$= 4.128, p<.001; Dose: P1: $F_{3,21}$= 3.390, p=.007; P2: $F_{3,21}$=10.784, p<.001; -P4: $F_{3,21}$=2.932, p=.05). Data are shown as mean +/- SEM.

Figure 16: Effects of quinpirole on choice behavior in the uncued task.

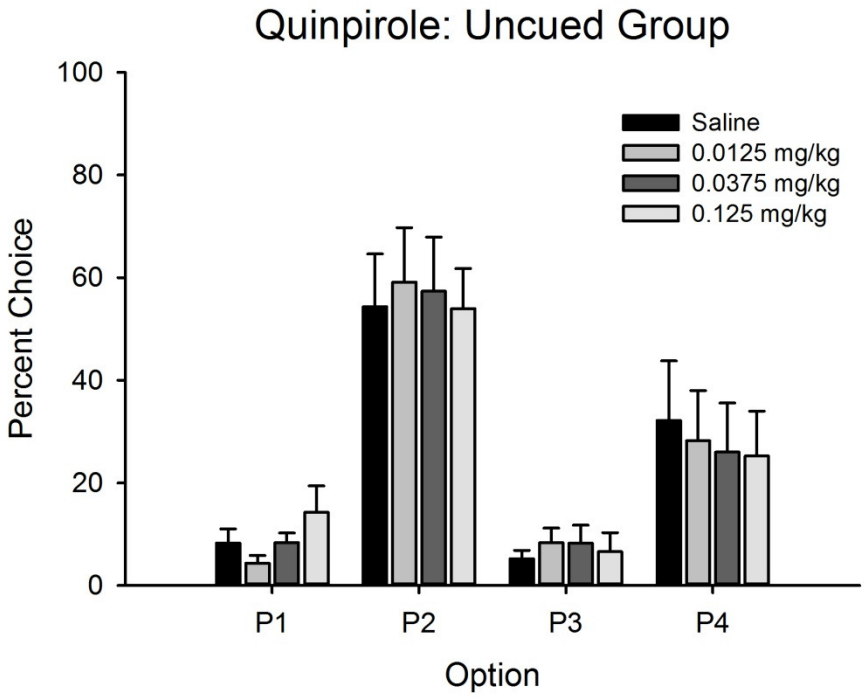

Figure 16: Effects of quinpirole on choice behavior in the uncued task. The uncued group did not show any change in choice preference in response to quinpirole (Uncued group: Dose x Choice: $F_{9,54} = 1.067$, NS). Data are shown as mean +/- SEM.

Figure 17: Effects of quinpirole on latencies.

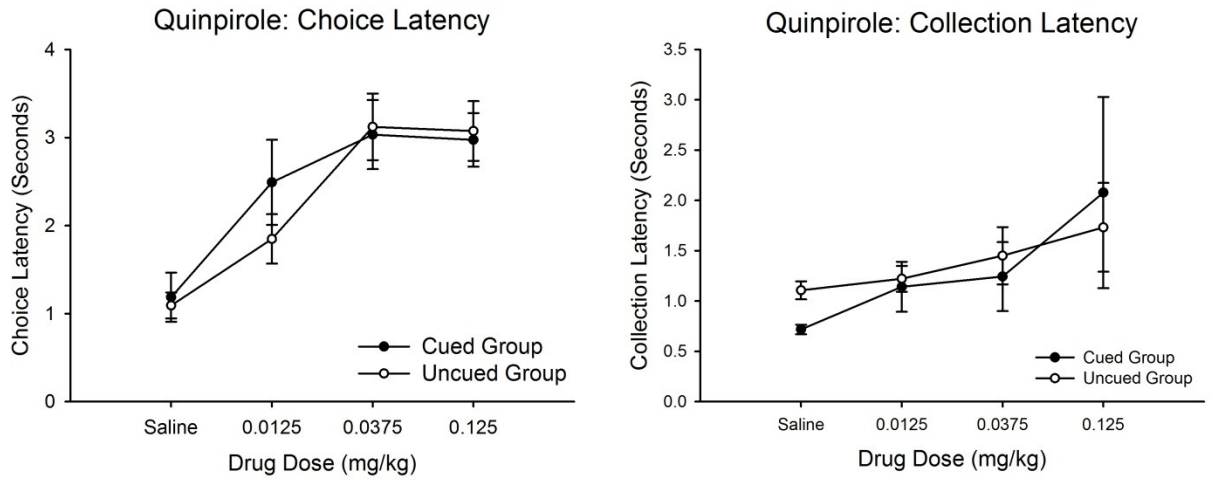

Figure 17: Effects of quinpirole on latencies. Quinpirole increased choice latency (Dose: $F_{3,39}$= 31.474, $p<.001$; Dose x Group: $F_{3,39}$= 1.156, NS) in both groups. It did not affect collection latency. Data are shown as mean +/- SEM.

Figure 18: Effects of quinpirole on premature responding.

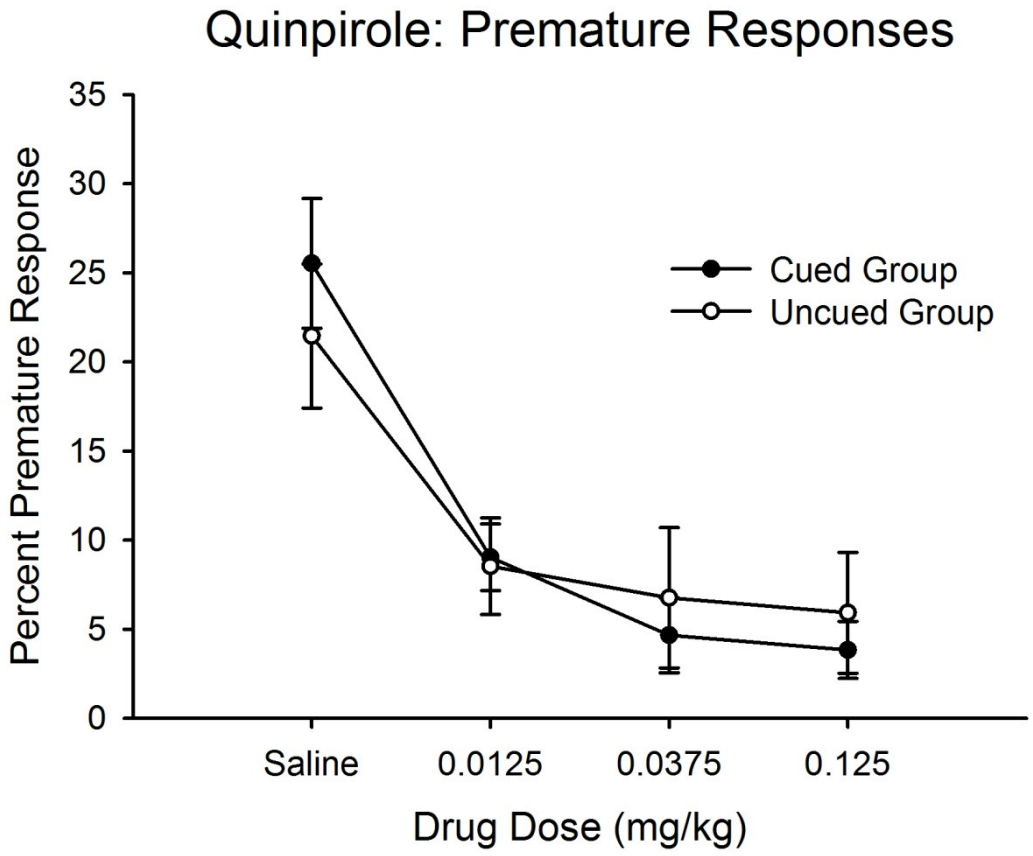

Figure 18: Effects of quinpirole on premature responding. Quinpirole decreased premature responses (Dose: $F_{3,39}$= 22.722, p<.001; Dose x Group: $F_{3,39}$=.391, NS). Data are shown as mean +/- SEM.

Figure 19: Effects of A-381393 on choice behavior in the cued task.

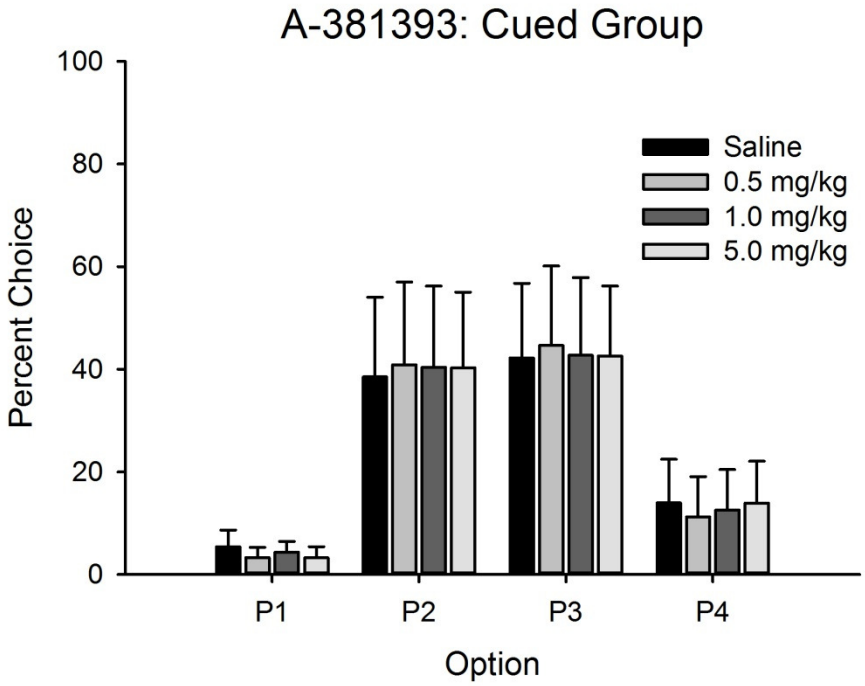

Figure 19: Effects of A-381393 on choice behavior in the cued task. A-381393, a selective D_4 receptor antagonist, did not significantly affect choice in either group (Figure 15, Figure 16Dose: $F_{3,39}$= .279, NS; Dose x Choice: $F_{9,117}$= .209, NS). Data are shown as mean +/- SEM.

Figure 20: Effects of A-381393 on choice behavior in the uncued task.

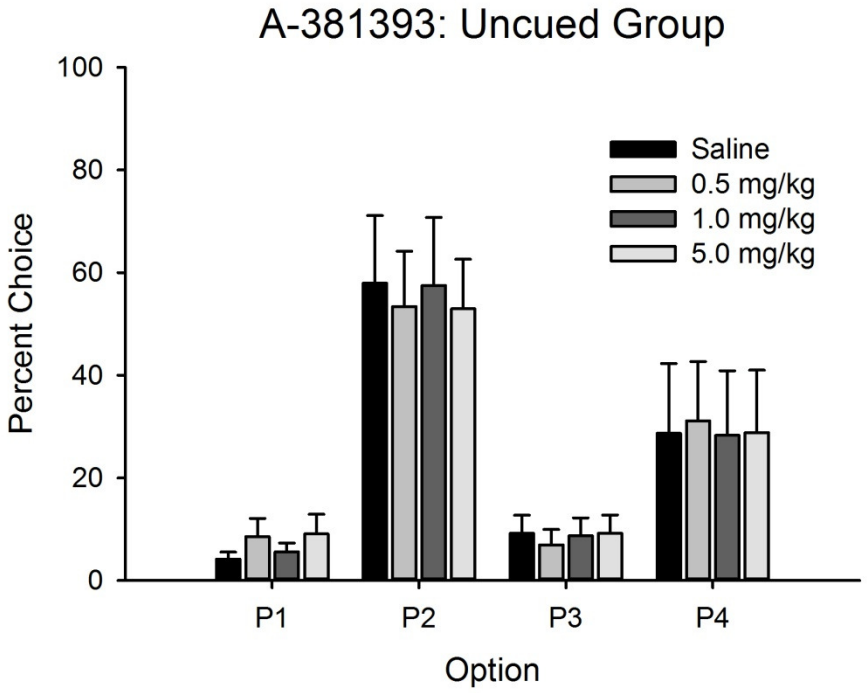

Figure 20: Effects of A-381393 on choice behavior in the cued task. A-381393, a selective D_4 receptor antagonist, did not significantly affect choice in either group (Dose: $F_{3,39}$= .279, NS; Dose x Choice: $F_{9,117}$= .209, NS). Data are shown as mean +/- SEM.

Figure 21: Effects of A-381393 on latencies.

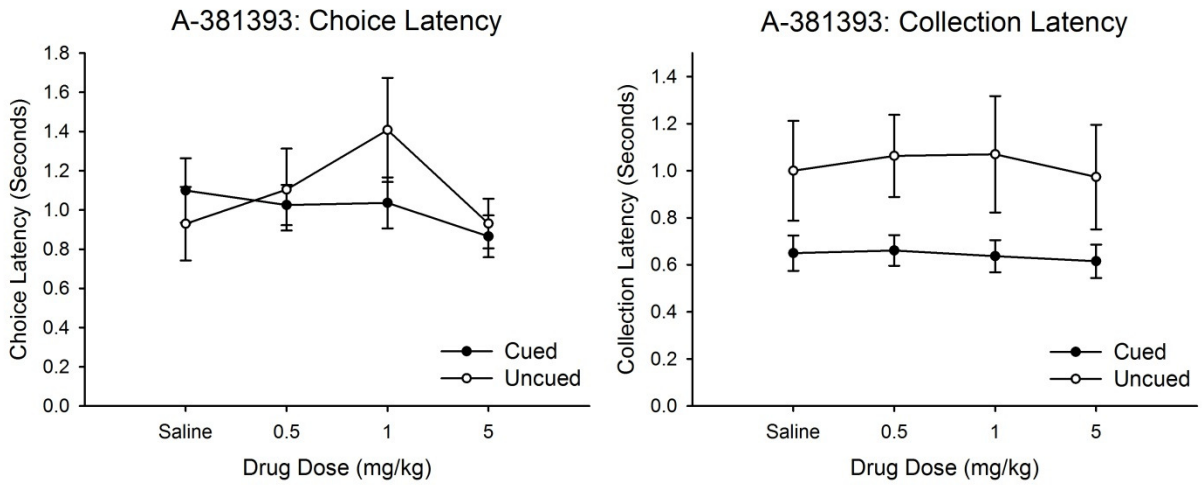

Figure 21: Effects of A-381393 on latencies. A-381393 did not affect latencies. Data are shown as mean +/- SEM.

Figure 22: Effects of A-381393 on premature responding.

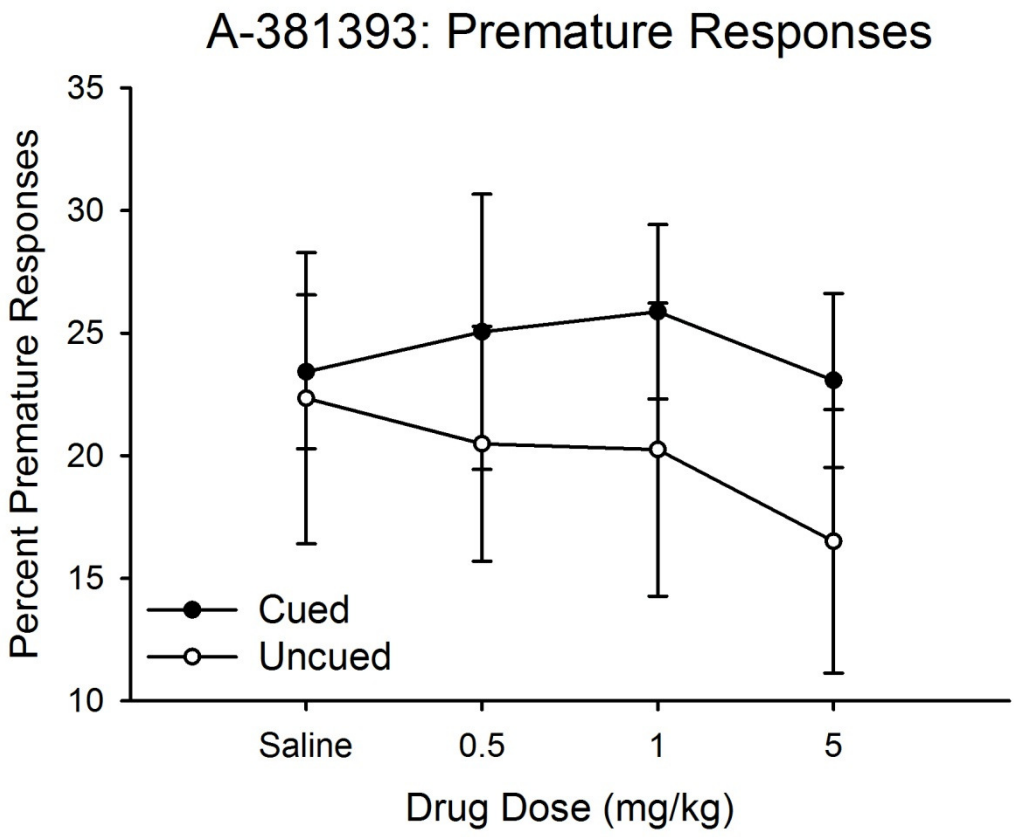

Figure 22: Effects of A-381393 on premature responding. A-381393 did not affect premature responding. Data are shown as mean +/- SEM.

Figure 23: Effects of PD-168077 on choice behavior in the cued task.

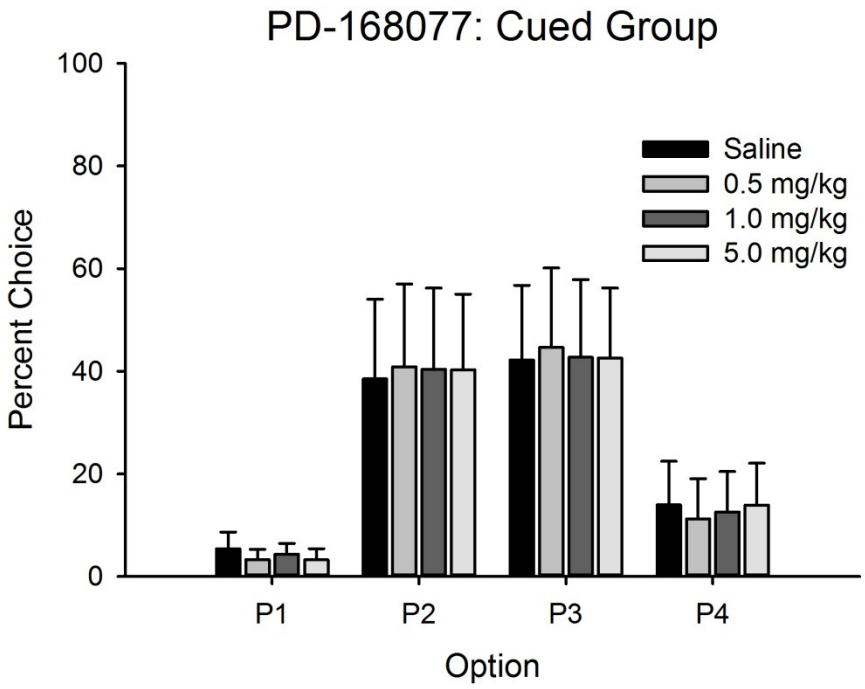

Figure 23: Effects of PD-168077 on choice behavior in the cued task. The D_4 receptor agonist PD-168077 did not affect choice behaviour in the cued group (Dose: $F_{3,39}$= .279, NS; Dose x Choice: $F_{9,117}$= .209, NS). Data are shown as mean +/- SEM.

Figure 24: Effects of PD-168077 on choice behaviour in the uncued group.

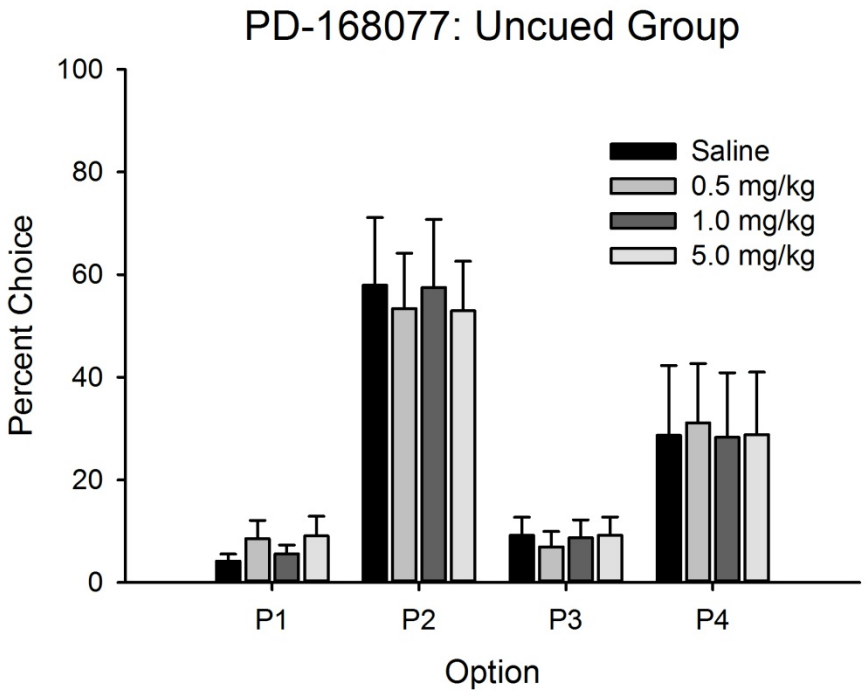

Figure 24: Effects of PD-168077 on choice behaviour in the uncued group. The D_4 receptor agonist PD-168077 did not affect choice behaviour in the uncued groups (Dose: $F_{3,39}$= .279, NS; Dose x Choice: $F_{9,117}$= .209, NS). Data are shown as mean +/- SEM.

Figure 25: Effects of PD-168077 on latencies.

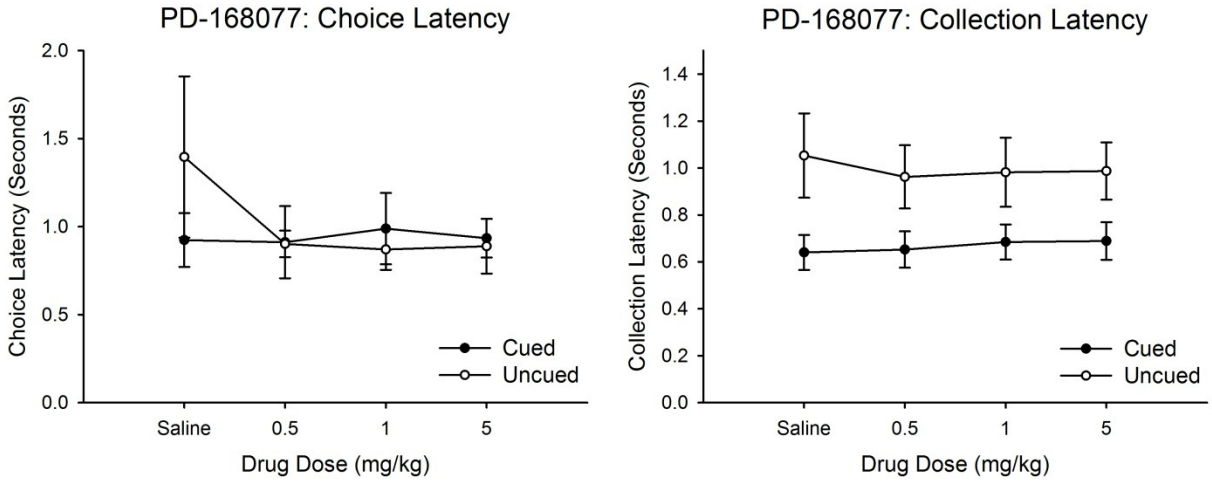

Figure 25: Effects of PD-168077 on latencies. The D_4 receptor agonist PD-168077 did not affect either choice latency or collection latency. Data are shown as mean +/- SEM.

Figure 26: Effects of PD-168077 on premature responding.

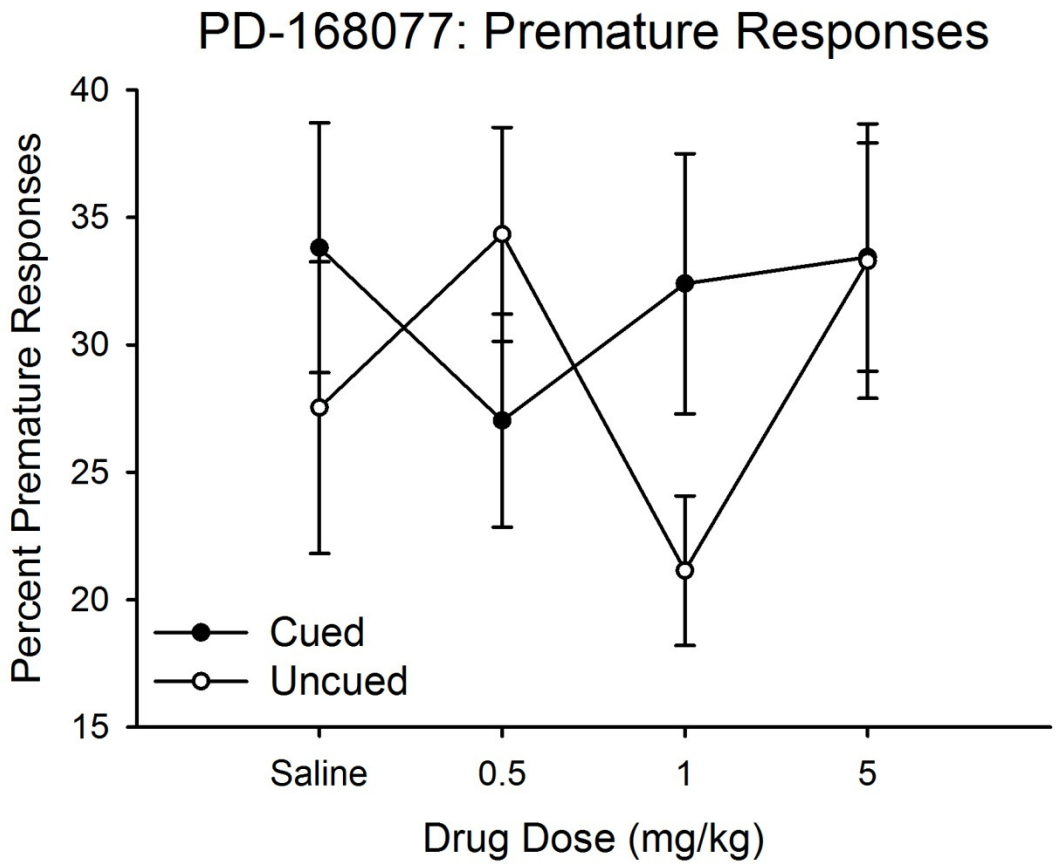

Figure 26: Effects of PD-168077 on premature responding. The D_4 receptor agonist PD-168077 did not affect premature responding. Data are shown as mean +/- SEM.

Discussion

Overview of findings

This work demonstrates that salient win-related cues are sufficient to shift behaviour away from an advantageous choice strategy and towards a riskier, disadvantageous one. This change in choice preference is not accompanied by an increase in motor impulsivity. Amphetamine increased choice of P1 in both the cued and uncued versions of the task, a result that is somewhat consistent with previous findings from the rGT (Zeeb 2009, Zeeb 2013). The D_2-like receptor agonist quinpirole decreased choice of P2 on the cued task, but not the uncued task, suggesting quinpirole's effects in guiding decision-making may be specific to the presence of salient cues. Eticlopride, A-381393 and PD-168077 did not appear to affect choice or most other behavioral measurements. We therefore conclude that win-related cues influence decision-making on this task and these effects may be mediated by activation of D_2-like receptors. These findings suggest the cued rGT is a valuable model with which to study how salient cues can invigorate maladaptive decision making, an important and understudied component of pathological gambling and substance use disorders.

Win-related cues bias decision making

Perhaps the most compelling finding from this work was that win-related cues were sufficient to bias animal's decision-making preference away from the most advantageous options and towards disadvantageous but heavily cued ones. Analysis of the score variable demonstrates animals in the cued conditions showed, on average, a slight preference for the disadvantageous options, while animals on the uncued task more strongly preferred the advantageous options. It was thus surprising the group by choice analysis returned a trend towards group differences ($p=.092$) but did not achieve statistical significance. Despite this null effect, visual inspection of

the average choice preferences of each group suggested animals in the cued group had lower choice of P2 (the most advantageous option), and higher choice of P3 (a disadvantageous option). When choice of each option was analyzed across groups, there was in fact a significant difference between groups in choice of P2 and a weak trend (p=.11) toward a difference in choice of P3. The null effect of group may stem from heterogeneity in the cued group. Disadvantageous choice preference came in two forms in the cued condition; animals in the cued group who preferred the disadvantageous options tended to have exclusive preference of either P3 or P4, and not a general preference for both. This pattern of behaviour led to a high degree of variability in the average choice of these options across the entire group. Therefore, while the null main effect of group across all choices was unexpected, individual differences in the specific nature of disadvantageous choice, in combination with the small sample size of this cohort, may have dampened our ability to detect a group by choice interaction. The addition of more animals to this sample may enhance our ability to detect a group by choice effect in the future.

Caveats and limitations

As this null effect demonstrates, a significant but easily remedied concern with regards to this data is the small sample it has been drawn from. Using a small sample may not provide enough statistical power to clearly delineate some of the more subtle effects of cues or pharmacological challenges. In previous experiments using the rGT, drug-dependent changes in choice behavior have sometimes been slight, and it is only the use of sufficiently large cohorts that provides the statistical power to determine these effects are not due to chance. Indeed, the cohorts described here (n=8, n=7) are half (or less) the size of the cohorts described in past publications using the rGT (see Zeeb 2009, Zeeb 2011, Zeeb 2013). Running additional animals on each task and repeating selected pharmacological challenges will increase the power of our

analysis and the reliability of our results. We are currently in the process of adding animals and anticipate the additional data will clarify the findings we have reported here.

An argument could be made that cues inhibit learning, perhaps by confusing or distracting the animal, and the difference in choice preference seen between conditions reflects an incomplete understanding of the task's contingencies rather than a fully-formed but disadvantageous decision-making strategy. This is possible, but several lines of evidence suggest it is not in fact the case. Were cues disruptive or inhibitory to learning, we would expect animals on the cued task to take longer to achieve behavioral stability. However, animals trained on both the cued and uncued versions of the task achieve stability at similar times, indicating their behavior is not driven by chance, randomness or simple sampling, but instead reflects an established and merely different choice preference. Furthermore, if animals on the cued task were somehow confused about the task's contingencies, we might expect them to commit more general performance errors. Their general aptitude is no different than that of animals on the uncued task; animals on both tasks have similar rates of premature responses and omissions (Figure 5). As they do not exhibit slower rates of learning nor commit more basic errors, we can take these data to support the conclusion that cues do not induce a learning deficit but simply drive different choice preferences.

Amphetamine and the rGT

An unexpected finding from this work is that amphetamine had similar effects on choice behavior on both versions of the task. An amphetamine challenge produced a dose-dependent increase in choice of P1 and a weak trend ($p=.15$) towards a decrease in choice of P2. The fact amphetamine induces a seemingly risk-averse profile on both versions of the rGT is counter-intuitive, given that amphetamine reliably increases responding for reward-related cues (Hill

1970, Robbins 1976) and has been found to increase choice of larger but delayed, or larger but probabilistic, rewards (e.g. Winstanley et al. 2004, St. Onge and Floresco 2009). However, a key difference between the rGT and these other behavioural tasks is that a failure to win is explicitly punished in the rGT by a signaled time-out, designed to convey "loss". This time-out is heavily cued by a flashing stimulus light. In addition to increasing the behavioural influence exerted by reward-paired cues, amphetamine also potentiates the ability of cues associated with aversive events, such as foot shock, to inhibit ongoing behaviour- a phenomenon known as conditioned suppression (Killcross et al. 1997). As such, amphetamine may make animals more sensitive to the punishment signal uniquely present in the rGT, therefore biasing animals towards the options associated with the lowest penalties. We therefore reasoned that, if reward delivery was also cued such that larger wins were associated with the most salient audiovisual stimuli present in the task, the direction of amphetamine's effects might switch to promoting choice of the high-risk high-reward decks. As noted above, this was not observed; amphetamine appears to have continued to bias animals towards P1 even in the cued version of the task.

D4 manipulations and choice behaviour

It is perhaps not surprising that D_4 manipulations had no effect on choice behaviour in this task, given the numerous reports of null behavioral effects of D_4 drugs (Oak et al 2000, Le Foll et al 2009). However, the null effects here do contrast our lab's findings on the rodent slot machine task (rSMT) in which the animal must correctly interpret of a series of cue lights as being indicative of a win or loss in order to make the best choice (Cocker et al 2014). In the rSMT, administration of a D_4 receptor agonist increased reward expectancy errors, such that animals responded to loss cues as if they were predictive of reward delivery, while antagonism of the same receptor subtype decreased these errors. These findings lead the authors to suggest "D_4

receptors play a critical role in attributing emotional salience to environmental stimuli and guiding response to these cues". It would follow that D_4 receptor manipulations may have effects on choice behavior in the cued rGT, as decision making on the task appears to be influenced by the salient win-related cues. The reason for this discrepancy is unclear, but it could relate to the divergent role of cues in each task or the intricacies of D_4's role in salience attribution. In the rSMT, cues are reward-predictive, whereas the cues in the cued rGT are reward-concurrent. These two conditions may be sufficiently distinct to where they rely on different neurocognitive processes with different neurobiological underpinnings. There is also some evidence (Lauzon et al 2009) that D_4 receptors exert their effects by modulating the salience of sub-threshold events. If D_4's role in decision-making is confined to those situations in which cues are not normally sufficient to modulate behavior, it may not extend to contexts like that of the cued rGT in which cues already have a demonstrable ability to influence decision making.

$D_{2/3}$ manipulations and choice behaviour

Quinpirole altered choice behavior on the cued rGT, but not the uncued version. Higher doses of quinpirole increased choice of P_1 and produced a near-significant trend towards increased choice of P_4 (p=.057), while decreasing choice of P_2. Consistent with previous work (Zeeb et al. 2009), these effects were not seen on the uncued version of the task, suggesting quinpirole's effects may be uniquely mediated by win-related cues. The potential involvement of D_2-like receptors on choice behavior in the cued but not uncued task is in line with findings pointing to a role for D_2 in reward-related behavior, particularly in the context of cues. Quinpirole increased reward expectancy errors on the rSMT, which prominently features reward-predictive cues (Cocker et al 2013). D_2-like receptors have been implicated in addictive disorders (Volkow 2003), compulsive behaviours (Johnson et al 2010), cue sensitivity and drug craving

(Heinz et al 2004) and behavioural motivation for reward (Chausmer et al 1997, Self et al 1996). Blunted D_2 activity in the striatum of human drug addicts is thought to be responsible for a reward-insensitivity phenotype, further supporting a hypothesized role for D_2 in the motivational effects of rewards. It has been suggested D_2 receptors are involved in mediating the attention paid to salient, reward-related stimuli, and dysfunction of this mechanism can lead to enhanced attention processing of these cues and subsequent increases in "wanting" or motivation to pursue cued rewards (Heinz et al 2004). D_2 agonism may therefore exert its effects by biasing attention towards the options paired with the most salient appetitive stimuli on the cued rGT, in spite of the deleterious consequences of pursuing these options.

These findings are all the more noteworthy when considered in the context of the relatively subdued effects of dopaminergic manipulations on choice behavior on the rGT. Contemporary understanding of dopamine's role in guiding reward-related behaviour encourages the expectation that rGT would be heavily mediated by dopamine, and dopaminergic manipulations would have robust effects. This has not been the case; with the exception of eticlopride and amphetamine, dopaminergic compounds have not affected choice behavior on the task. Furthermore, amphetamine's effects do not appear to be dopaminergic; selective dopamine antagonists do not attenuate amphetamine's effects on choice behavior (Zeeb et al 2013). Concurrent administration of GBR 12909 (a selective dopamine reuptake inhibitor) and a selective noradrenaline reuptake inhibitor replicated amphetamine's effects on choice behavior, providing further evidence that these changes in choice behaviour result from something more complex than a simple increase in dopaminergic activity, and may be due to the interplay of several monoamines (Baarendse et al 2012) Dopamine's role in decision making may be limited on the rGT because of the complex interplay of reward size, punishing timeouts and differing

probabilities of reinforcement (Baarendse et al 2012). It is therefore interesting the addition of cues seems to have made the task dopamine dependent, and further research should be pursued in order to explore the role of dopamine in this form of cue-mediated decision making.

Choice of P3 vs P4 in the cued rGT

An unexpected finding from this work was the similarity in preference for P3 and P4 amongst animals performing the cued task. We expected the more salient cues of P4 would bias attention toward that option, but animals in the cued condition chose P3 and P4 at similar rates, suggesting they perceive these options as more or less equivalent. This finding was initially confusing, as P4 is associated with both larger food rewards and more complex cues (albeit larger and more frequent punishments). After looking through video of several (n=4) animals performing the cued task, we noticed animals orient towards the food hopper as soon as a win is signaled, which puts one group of win-related out of their field of vision. While this represents only one component of the complex win-related audiovisual cue sequence, much of the variability between P3 and P4's visual cues are accounted for by this group of lights. Animals exhibiting this pattern of behavior are primarily oriented towards the flashing food tray light during the win sequence, which does not differ between P3 and P4. Therefore, it may be that animals behaving in this manner do not experience the visual cues associated with P3 and P4 as sufficiently distinct to definitively bias choice towards either option; this may contribute to the similar rates of choice between the two.

This observation suggests an area for exploration in future versions of the task. The visual-spatial orientation of cues and reward in the cued rGT is dissimilar to human gambling, where the winning stimuli are often visually proximate to the reinforcer (such as money or chips). Designing the win cues in such a way that they remain in the animal's visual field while

they are collecting their reward will make the task more environmentally valid and could potentially increase choice of P4. This could be accomplished by either moving the cues closer to the food tray or delaying the dispensation of reward until the cues have played. These are preliminary ideas, but the issue could be given some thought in future attempts to manipulate choice of P4 in subsequent experiments.

Individual differences and the cued rGT

Despite these caveats, the cued rGT holds great promise. One of the most encouraging elements of the cued rGT is its ability to distinguish individual differences in choice behavior. The rGT has returned relatively consistent patterns of choice behavior across subjects, whereas there is considerable individual variability on the cued rGT. While consistency is a benefit for studying the effects of pharmacological and surgical manipulations in small cohorts, it makes it difficult to study individual differences in choice preference. As patterns of maladaptive decision-making are thought to be critical to the development of addictive disorders, the cued rGT's ability to parse cohorts into groups of advantageous and disadvantageous decision makers suggests that the task may enable experimental exploration of the processes that guide optimal vs. suboptimal choice. While current cohorts are too small to reliably divide into behaviourally distinct subgroups, there is a trend towards divergent patterns of decision making in the cued group. It appears some animals are relatively unaffected by the cues and able to maintain a highly advantageous choice strategy, while the appetitive properties of the cues encourage others towards an inappropriately risky choice preference. Recurrent use of this task may result in the emergence of distinct subgroups defined by their relative preference for the saliently-cued options; the study of these groups may reveal behavioural and neurobiological differences between cue-driven risky decision makers and those resistant to the motivating effects of cues.

Conclusions

The data presented here demonstrates a role for salient win-related cues in guiding decision making, and points to a possible role for D_2 receptors in mediating this behavior. These findings contrast previous work on the rGT that did not find a significant role for dopamine in guiding choice behavior, and suggests cue-paired decision making may recruit unique neurobiological processes. Further work with the task may clarify the effects of these pharmacological manipulations and provide the opportunity to explore individual differences in cue-mediated decision making.

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