Investigating the neurobiological underpinnings and structural characteristics that contribute to biased decision making.

by

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Abstract
Gambling disorder (GD) and other forms of behavioural and substance addictions are characterized by deficits in decision making and impulsivity. Animal models of behaviour allow researchers to study specific components and features of biased decision making and elucidate the neural circuitry that underpins these biases. Our laboratory has developed several animal models which each examine a specific subfacet of decision making thought to contribute to GD. The rat Gambling Task examines risky decision making by posing a choice between small, safe rewards and larger but more risky options. The rat Betting Task examines a specific choice bias termed the “escalation of commitment” bias, in which humans show an increasing aversion to uncertain wagers as the amount at stake goes up. In order to examine the effect of cues on choice behaviour, we developed the Cued rat Gambling Task, which offers the same choice options as those on the rat Gambling Task, but pairs wins on the risky options with salient cues. The presence of salient environmental stimuli can influence choice behaviour, and may make important contributions to the maintenance and severity of GD.

As described in this dissertation, work with these tasks has clarified the environmental, pharmacological and regional contributions to GD-like decision making. The development of the Cued rat Gambling Task demonstrated that the addition of salient cues is sufficient to drive a riskier, more disadvantageous choice preference than that demonstrated on the uncued rat Gambling Task. Disrupting orbitofrontal cortex function while the animal is learning the Cued rat Gambling Task promotes the development of a more optimal choice strategy, perhaps due to the region’s roles in cognitive flexibility and subjective valuation. Choice biases on the rat Betting Task are also ameliorated by inactivation of the orbitofrontal cortex, but not inactivation of other prefrontal regions. Furthermore, the nucleus accumbens core, but not shell, may guide the development of a risk-averse choice strategy on the rat Gambling Task. In sum, the work in
this dissertation clarifies the motivational role of cues in gambling and other disorders of addiction, and elucidates the neural circuitry that underpins choice biases.
Lay Summary
People suffering from Gambling Disorder are unable to control their gambling, in much the same way that people with a substance addiction cannot stop using drugs or alcohol. Researchers who are interested in gambling and addiction-like behaviour benefit from using animal models of these behaviours, because these models allow researchers to employ invasive techniques that are not possible in humans. These techniques include using novel drugs and shutting down different regions of the brain, which provide valuable information about the neuroanatomy and neurochemistry involved in gambling and addiction-like behaviour. The research in this dissertation studied behavior on two existing animal models of decision making, and developed a third to examine how salient cues affect decision making. Our results clarify how certain regions of the brain and certain neurochemicals contribute to decision making and biased choices, and how salient cues can change decision making.
Preface
This thesis is an original intellectual produce of the author, Michael Barrus (M.M. Barrus), with the guidance and mentorship of Dr. Catharine Winstanley (C.A. Winstanley). Experimental work was conducted in the laboratory of C.A. Winstanley at the University of British Columbia, within the Department of Psychology. Experiments were designed by M.M. Barrus and C.A. Winstanley.

Experiment one (chapter 3) has been published as “Dopamine D3 Receptors Modulate the Ability of Win-Paired Cues to Increase Risky Choice in a Rat Gambling Task” by M.M. Barrus and C.A. Winstanley (2016), Barrus, M. M., & Winstanley, C. A. (2016). Journal of Neuroscience, 36(3), 785–794. http://doi.org/10.1523/JNEUROSCI.2225-15.2016. M.M. Barrus assisted with the development of the task, collected behavioural data, performed data analysis, co-wrote the manuscript, and interpreted the results. C.A. Winstanley developed the task, co-wrote the manuscript and interpreted the results.

Experiment two (chapter 4) is being prepared for publication by M.M. Barrus, N.Z. Gagnon, M. Tremblay, G. Betts and C.A. Winstanley. M.M. Barrus collected behavioural data, performed data analysis, co-wrote the manuscript, and interpreted the results. N.Z. Gagnon collected behavioural data. M. Tremblay assisted with surgery. G. Betts performed histology. C.A. Winstanley co-wrote the manuscript and interpreted the results.

Experiment three (chapter 5) is being prepared for publication by M.M. Barrus, L. Mortazavi, T.J. Hynes, B.A. Hathaway, G. Betts and C.A. Winstanley. M.M. Barrus collected behavioural data, performed data analysis, co-wrote the manuscript, and interpreted the results. L Mortazavi collected behavioural data. T.J. Hynes assisted with surgery and brain collection. B.A.
Hathaway and G. Betts assisted with brain collection. C.A. Winstanley co-wrote the manuscript and interpreted the results.

Experiment four (chapter 6) has been published as “Inactivation of the orbitofrontal cortex reduces irrational choice on a rodent Betting Task” by M. M. Barrus, J. G. Hosking, P. J. Cocker, and C.A. Winstanley, C. A. Neuroscience.

http://doi.org/10.1016/j.neuroscience.2016.02.028. M.M. Barrus collected behavioural data, performed data analysis, co-wrote the manuscript, and interpreted the results. J.G. Hosking and P.J. Cocker assisted with surgery. C.A. Winstanley developed the task, co-wrote the manuscript and interpreted the results.

All animal testing was performed in accordance with the Canadian Council on Animal Care (CCAC) and received ethical approval by the University of British Columbia Animal Care Committee, certificate numbers A13-0011 (behavioural protocol) and A15-0011 (breeding protocol). Adeno-associated virus delivery was performed in accordance with approved standard operating procedure ACC WINSTANLEY CAW116. All procedures are standard for working with rAAVs classified as Risk Group 1, and have been approved by the UBC Biosafety Committee (protocol B15-0027).
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Dedication

To Chloe. I wish we could have celebrated this together. I’ll carry you with me when I do.
Chapter 1: Introduction

Gambling is a common recreational pastime that can lead to debilitating and compulsive behaviour for some users. While most individuals are able to gamble within reasonable limits, some 12.5% of the general public demonstrates sub-clinical problem gambling, and 2.5% meet the criteria for Gambling Disorder (GD), a DSM-V recognized behavioural addiction characterized by a loss of control over gambling (Cunningham-Williams et al., 2005). Despite GD’s prevalence and social and individual costs, the neurobiology of gambling behaviour is not well understood. This lack of insight has thus far limited treatment of the disorder. Laboratory-based models of gambling behaviour are therefore useful in that they allow researchers to isolate the cognitive and neurobiological processes implicated in gambling. Analogues of human gambling paradigms with strong face, construct and predictive validity can then be designed for use with non-human laboratory animals, thereby enabling researchers to determine the causative role of particular brain areas or neurotransmitter systems in maladaptive gambling behaviour (Winstanley & Clark, 2016; Cocker & Winstanley, 2015; for discussion). Establishing such models is an important research priority in the field as they have the potential to inform our understanding of the factors that contribute to gambling behavior, the underpinning neurobiology of GD, and the potential to catalyze the development of pharmacological treatments for GD.

Work in the Winstanley lab has established several animal models that each capturing distinct dimensions of gambling-like behaviour. Among these are the rat Betting Task (rBT), the rat Gambling Task (rGT), and the Cued rat Gambling Task (CrGT). While all of these models offer a choice between options associated with different probabilities of reward, they vary in the specific components of gambling behaviour they attempt to address.

1.1 Choice biases and the rBT
The rBT was designed to measure one commonly-observed decision-making heuristic in rodents, namely “escalation of commitment” in which subjects become more risk averse as the stakes increase, even if the odds of success remain constant (Trepel, Fox, & Poldrack, 2005). In the rBT, the animal is presented with a choice between two options of equivalent expected value, such that sucrose pellet reward on one option is guaranteed while the other has a 50% chance of double the amount of reward or nothing. Past work has shown that a subset of animals (which have been termed ‘wager sensitive’) adopt an irrationally “risk-averse” choice preference in which they shift their choice away from the uncertain option as the bet size grows larger. Given that GD is associated with increased risky choice on laboratory paradigms such as the Iowa Gambling Task (IGT) (Goudriaan, Oosterlaan, de Beurs, & van den Brink, 2005), it may seem counterintuitive to associate this superficially risk-averse wager sensitivity with GD. However, wager sensitivity on the rBT does not benefit the participant in the way that risk aversion would in the context of the IGT, where a risk averse strategy would help the participant avoid the more uncertain options that are ultimately disadvantageous (Bechara, Damasio, Damasio, & Anderson, 1994; Bechara, Damasio, Damasio, & Lee, 1999). Furthermore, wager sensitivity is dissociable from an animal’s preference for uncertainty; amphetamine increases choice of the uncertain option in wager sensitive animals, without impacting the degree to which those animals are wager sensitive (Cocker, Dinelle, Kornelson, Sossi, & Winstanley, 2012). Switching to the certain option as wager size increases does not help the animal avoid risk because each option is mathematically equivalent, nor does it confer any other benefit to the animal (Cocker et al., 2012).

Taken together, this evidence suggests that wager sensitivity is less like risk aversion or uncertainty aversion than it is like an irrational choice bias. Choice biases are a well-recognized
component of GD; for example, “Interpretive Biases” and “Gambling Expectancies” are two of the components assessed by the Gambling-Related Cognitions Scale (Raylu & Oei, 2004) which correlates with the severity of GD. These biases range from distorted beliefs whereby individuals overestimate their likelihood of winning (Ladouceur& Walker, 1996), to the ‘illusion of control’ in which individuals assess their performance in games of chance as being contingent on their ‘skill’ at the game, rather than being determined the stochastic nature of the game itself (Ladouceur, Sylvain, Letarte, Giroux, & Jacques, 1998). These and other biased beliefs are recognized as being central to the etiology of GD, and may represent the cognitive mechanism by which recreational gambling progresses to disordered or pathological gambling (Clark, 2010). The rBT therefore represents a valuable tool for examining one form of these biases in an animal model.

Wager sensitivity shares features of several recognized biases in decision making that could contribute to GD. For one, it is proposed to resemble “myopia for future reward”, where animals shift their choice to the certain option because they are intolerant of the inconsistent reinforcement delivered by the uncertain option (Cocker et al., 2012). This bias is somewhat reminiscent of that examined by delay discounting paradigms, where subjects shift their preference towards smaller yet immediate gains as the delay to a larger reward increases (Ainslie, 1975; Dixon, Marley, & Jacobs, 2003). This bias in favor of immediate reward is observed in subjects with substance use disorders (Bickel, Yi, Landes, Hill, & Baxter, 2011) and GD (Dixon et al., 2003), and has even been proposed to be the essential cognitive mechanism that guides the development of addiction (Bechara, 2005). At its essence, wager sensitivity might alternatively be understood as a subjective choice bias; given that rational, unbiased choice on the rBT would be indifference between the two options, a preference for one over the other
reflects a subjective assessment that one option is better than the other. Such subjective assessments may underlie myriad forms of poor decision making where individuals know the objective values of the choices before them, and yet fail to choose the best option. Research employing the rBT can provide thus insight into the neurobiology that drives this bias.

Work with the task has demonstrated a relationship between decreased D_{2/3} receptor expression in the striatum and wager sensitivity (Cocker & Winstanley, 2015). Given that aberrations in striatal dopamine have been linked to addiction vulnerability (Volkow et al., 2002), this finding suggests that rBT performance may be dependent on neurobiological substrates that are recruited in both substance use disorders and behavioural addictions such as GD. The logical continuation of this research would be the examination of other regions that are thought to be important to behavioural and substance addictions. Potential targets would include the ventromedial prefrontal cortex (vmPFC), a region which appears to be involved with both subjective preference (Kringelbach & Radcliffe, 2005) and substance abuse (Volkow & Fowler, 2000). Despite the benefits of the model, work with the rBT considers only a limited scope of gambling-like behaviour, and the development of additional tasks that examine other components of gambling would complement this research and further our understanding of the neurobiology of GD.

1.2 Risky decision making and the rGT
The rGT is a model of decision making in which animals are given a choice between several options associated with varying schedules of reward and punishment. This model is based on the Iowa Gambling Task (IGT), one of the most widely used cognitive tasks in humans that assesses decision-making processes similar to those recruited during gambling behaviour (Bechara et al., 1994). Although ostensibly designed to capture “real-world” decision-making in
which all options could lead to both gains and losses according to initially ambiguous odds, it has been used as a proxy for gambling largely due to its strong superficial resemblance to the act of gambling. In the IGT, human participants must choose between decks of cards, each of which is associated with different schedules of risk and reward, in order to maximise the amount of money or points earned. Two of the decks (decks A and B) are associated with sizeable wins but also disproportionately larger 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. Work with the IGT has demonstrated impairment in a number of clinical populations including pathological gamblers (Verdejo-Garcia et al., 2007; Goudriaan et al., 2005). While there are numerous aspects of problematic gambling behaviour that are not captured by this task (see Bechara et al., 1994 for discussion), there is no doubt that work with the IGT has made a significant contribution to our understanding of decision-making under conditions of risk and ambiguity. The rGT was therefore developed as a rodent analogue of the IGT in order to capture these elements of disordered gambling; having such an animal model would enable research into the neurobiological correlates of task performance (Potenza, 2009; Winstanley, 2011a).

In the rGT, animals are allowed to choose between four options, signaled by illumination of four response apertures, loosely analogous to the four decks of cards used in the IGT in that each is associated with unique schedules of food reward or “time out” punishment (Zeeb, Robbins & Winstanley, 2009). As is true of the IGT, the best strategy on this task is to favor options associated with smaller rewards but also smaller punishments—this more conservative approach leads to the steady accumulation of the greatest amount of reward over time. In
contrast, a preference for these 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 during the course of a session. 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. Most rats acquire the optimal strategy readily, and such decision-making appears to depend on similar neural circuitry as is implicated in performance of the IGT; specifically, choice on the rGT appears to be mediated by the medial prefrontal cortex, orbitofrontal cortex, and basolateral amygdala (Paine, Asinof, Diehl, Frackman, & Leffler, 2013; Zeeb & Winstanley, 2011; Zeeb & Winstanley, 2013). These regions are also implicated in choice on the IGT (Bechara et al., 1999; Fellows, 2004). However, these are not the only regions that might mediate rGT performance. The nucleus accumbens appears to make extensive contributions to motivated behaviour (Floresco, 2015), and represents another target for investigation.

1.3 Salient win-related cues and the CrGT
While the rGT provides valuable insight into cost/benefit decision making, there are significant elements of real-world gambling that are not addressed by this model or others. To our knowledge, little work had been done evaluating the role of salient cues in modulating cost-benefit decision making in either human or animal models. This is a potentially rich area of research; real-world gambling is rife with salient cues, and their influence on gambling behaviour may be significant.
Past research on the behavioural influence of cues has primarily emphasized the prominent role of drug-related cues in substance addiction and abuse (Childress et al., 1993). Drug-related cues can be anything the user associates with the drug-taking experience, be that individuals with whom the user takes drugs, locations in which the user commonly takes drugs or drug-associated paraphernalia such as pipes or syringes. After repeated pairings of these people, places and things with the drug-taking experience, these formerly neutral stimuli come to predict the delivery of reward and may even take on the motivational properties of the reward, promoting drug-seeking behaviour and conditioned responses. Drug-associated cues such as paraphernalia and location can induce powerful craving and arousal states (Childress et al., 1993); exposure to smoking related cues increases subjective craving for cigarettes (Carter & Tiffany, 1999), while exposure to alcohol related cues increases subjective craving for alcohol (Schulze & Jones, 1999). The degree of attentional bias towards these cues can distinguish between abusers and non-abusers/non-users, and among users, substance-related bias tends to be proportional to the quantity and frequency of use (Robbins, n.d.).

The behavioural impact of cues is not limited to substance use and abuse. Highly salient win-associated cues are a significant component of human gambling, and may play an important role in modulating gambling behaviour. Similar to the relationship between drug use and its associated cues, exposure to gambling cues can induce craving in problem and frequent gamblers (Kushner et al., 2007). Problem gamblers appear to be more cue-sensitive than non-problem gamblers; for instance, adolescent pathological gamblers reported being more attracted by music, lights and noises produced by slot machines than non-pathological adolescent gamblers (Griffiths, 1990). Problem gamblers show greater interference effects of gambling-related words in a Stroop task as compared to healthy controls, suggesting that problem gamblers are less able
to shift their attention away from gambling-related stimuli. Attentional bias towards these cues may play a critical role in the transition from recreational to problem gambling (van Holst et al., 2012).

Despite these similarities, there may also be important differences in the roles that cues play in substance versus gambling contexts. Similar to drug cues, gambling cues are associated with rewards (in this case, monetary), or the possibility of rewards. However, in the case of gambling cues are linked with rewards at multiple levels. Broad contextual cues, such as red lights, casino sounds and appearance of gambling tables and machines, are not specifically associated with outcomes, yet signal the possibility of a reward if gambling is initiated. These seem phenomenologically most similar to drug cues. Anticipatory cues, such as reel spins and accompanying music, signal the possibility of an imminent reward in a given play. Outcome-specific cues, such as flashing lights and sounds of tumbling coins of the slot machine when a win occurs, are concurrent with and symbolic of monetary rewards and hence might themselves help reinforce and maintain gambling once it has already been initiated. Whereas other research has posited that sound serves as an occasion setter or discriminative stimuli that essentially sets the stage for other stimuli to modulate gambling behaviour (Griffiths and Parke, 2005), some have suggested that win-associated cues are second-order conditioned stimuli, which become rewarding in their own right (Dixon et al., 2014). This distinction is subtle but important. Again, describing salient cues such as win-related lights and sounds as mere occasion-setters relegates them to a supporting role in maintaining disadvantageous behaviour, rather than a driving force with direct influence on decision-making. They have frequently been described as the former; lights and sounds of fruit machines have been characterized as “psycho-structural… characteristics” that serve as “gambling inducers” (Griffiths, 1993), serving to “create an
atmosphere which is probably conducive to gambling” (Caldwell, 1974). In contrast, Dixon et al.’s work regards gambling-related stimuli as having a function similar to that of drug-related CSs, in that sound is capably of directly influencing disadvantageous gambling behaviour.

Different types of gambling cues (contextual, anticipatory, outcome-specific) may influence the gambler’s experiences and behaviour in different ways—a possibility that has not yet been comprehensively studied, but appears to be supported by at least some evidence. Though this research is in its infancy, the handful of existing studies suggest that contextual gambling cues affect subjective experiences and energize behaviour of the player, whereas outcome-specific (win-associated) cues additionally affect and distort gambling-related cognitions. Thus, ambient cues (red lights, casino sounds) that were not specifically associated with outcomes on the IGT had a positive effect on mood and speeded up reaction times to make choices following losses, but had no effect on choice behaviour (Brevers et al., 2015). Higher tempo of background music increased the speed of betting in a virtual roulette game, especially when combined with ambient red light, but did not affect bet size or the amount spent (Dixon et al., 2007). Though the effects of anticipatory gambling cues remain mostly unstudied, one experiment found that that sequential presentation of symbols on the different reels may be more reinforcing to the players than simultaneous presentation of the symbols on all the reels, as sequential presentation increased the number of games played (Ladouceur & Sevigny, 2002); however, varying the duration of the reel spin did not affect any aspect of gambling behaviour (Sharpe et al., 2005).

Unlike contextual cues, outcome-specific cues appear to affect play-related cognitions. The presence versus absence of specifically win-associated auditory cues —jingles varying in length and intensity as a function of win size—not only resulted in increased arousal (measured
via galvanic skin responses and self-report) and higher preference ratings for the cued version of the task, but also led the subjects to overestimate their frequency of winning (Dixon et al., 2014). Other evidence comes from studies of win-associated audiovisual cues that slot machines commonly present during “wins” that actually fall short of the amount wagered—in other words “losses disguised as wins” (LDW) (Dixon et al., 2010, 2015). Such audiovisual “disguise” proves compelling: LDWs resulted in indices of physiological arousal that were more similar to those produced by genuine wins than those produced by frank losses. Sounds accompanying LDWs, in their own right, had a significant impact on subjects’ impression of winning or losing: when LDWs were accompanied by winning sounds, players miscategorized the majority of these trials as wins and overestimated their overall frequency of winning; when LDWs were accompanied by losing sounds, both categorization and recall of winning frequency were considerably more accurate. Gambling-related cognitive distortions are believed to play an important role in driving pathological gambling (Clark, 2010). Therefore, the demonstrated effects of outcome-specific cues on cognitive variables raise the possibility that these cues could thereby help drive disadvantageous gambling-related choices and behaviour. To the best of our knowledge, this possibility has not yet been tested in humans, and the effects of outcome-associated cues on human choice behaviour remain unstudied. This area deserves more attention. Research with both human and animal models with careful manipulation of cues at every level would provide valuable insight that could ultimately inform prevention and treatment of disordered gambling. Further, given the sophistication of cues in gambling and gaming, systematic study of these cues and their effects could produce new insights regarding the role of cues in addiction more generally, which may have escaped recognition with the focus on the apparently simpler drug cues.
While the value of this human gambling research is self-apparent, the use of animal models provides insight that complements and expands on the human literature. Examining the behavioural influence of cues in rodent models provides more explicit neurobiological information and allows for manipulations that are not possible in human subjects. While the research into gambling-specific effects of cues is more limited (if not non-existent) in animal models, several established animal paradigms do investigate the ability of CSs to affect behaviour. These models differ in both structure and their mechanistic explanations for how cues guide behaviour; several of the most prominent are reviewed below.

Sign-tracking (ST) is a model of cue motivated behavior that has its roots in some of the oldest research on salient cues—that of Pavlov and his dogs. Pavlov’s seminal research demonstrated that some animals began to treat the stimuli predictive of reward as though it were the reward itself. He wrote ‘‘...the animal may lick the electric lamp (that is predictive of food), or appear to take the air into its mouth, or to eat the sound, licking his lips and making the noise of chewing with his teeth as though it were a matter of having the food itself’’ (Pavlov, 1927). Approach to and engagement with the cue suggested that it had taken on motivational properties of its own, and was not merely predictive of reward for some animals but rewarding in and of itself. This sort of engagement with the conditioned stimuli (CS) has since been well-documented in the literature; pigeons will peck at a cue light that predicts reward delivery, even though food delivery is not contingent on any instrumental response (Brown & Jenkins, 1968), while raccoons trained to deposit a token to receive a food reward treat the token as though it were food itself, washing it and gnawing on it for extended periods of time despite the fact that these behaviours prevent the acquisition of the food itself (Breland & Breland, 1961). In each of
these examples, it appears that the reward-predictive cue acquired great incentive value of their own, sufficient to distract at least some of the animals from the unconditioned stimuli (US).

Researchers have posited that the inclination to approach the reward-associated cue or “sign” over the reward itself represents a misattribution of salience that may be a marker for vulnerability to a host of behavioural disorders, including addiction (Flagel, Akil, & Robinson, 2010). In essence, ST tasks provide a measure of the ability of salient reward-related cues to gain control over behaviour, roughly analogous to processes seen in the maintenance and reinstatement of drug addiction. Work with these tasks have demonstrated, among other findings, increased sensitivity to cocaine-induced plasticity in sign-trackers (Flagel et al., 2008), distinct alterations in the dopamine system in sign-trackers and goal-trackers (Flagel et al., 2007), and elevated corticosterone in sign-trackers relative to other groups (Tomie et al., 2000, Tomie et al., 2004). ST has thus proved its worth in examining specific addiction related behavioural profiles, and work with the model is providing valuable insights into individual attributions of incentive salience and the “misbehaviour of organisms” (Breland & Breland, 1961).

Pavlovian to Instrumental Transfer (PIT) is another model of the behavioural impact of cues. PIT examines the ability of CSs associated with an outcome to invigorate instrumental responding for that outcome, even when there is no formal association between CS and instrumental responding. In PIT procedures, subjects learn two distinct contingencies. The first is a simple classical conditioning procedure in which the non-contingent delivery of a reinforcer is paired with a stimulus. Importantly, reinforcement is not dependent on any response from the subject. The second is an instrumental responding procedure where the subject must execute some behaviour in order to receive the same reinforcer (i.e. there is a causal relationship between the animal’s behaviour and the delivery of reward). The testing period then takes place in
extinction conditions, where the instrumental response is not rewarded, in order to see if presentation of the reward-paired stimuli invigorates engagement in the previously reward-paired action.

The ability of CSs to encourage responding for an US in PIT paradigms probably reflects the CSs’ ability to produce a general increase in motivation. PIT appears to provide reliable evidence that CSs can produce a general increase in motivation for desirable USs, suggesting one method by which cues can come to influence behaviour. This phenomenon has also recently been described in human subjects, and stronger PIT observed in individuals that exhibit greater sign-tracking to a reward-paired cue (Garofalo, Pellegrino, & Clark, 2015).

Tests of conditioned reinforcement (CRf) may look methodologically quite similar to PIT- again, the CS is first classically conditioned to reward delivery. However, the subsequent CRf test then determines the degree to which rats will perform a novel response, such as lever-pressing, that is reinforced solely by the CS. Thus, in contrast to PIT, presentation of the CS is entirely contingent on the animals’ behaviour (Robbins, 1978). The process of CRf is thought to underlie second-order schedules of reinforcement of drug self-administration that are typically used to assess drug-seeking rather independent of drug-taking (Arroyo, Markou, Robbins, & Everitt, 1998; Ciano & Everitt, 2005). In such paradigms, animals initially make a single response to receive an infusion of an addictive substance, such as cocaine, paired with an audiovisual CS, such as a light or tone. Over successive iterations, an association is therefore formed between experience of the drug and the CS. The power of this association is so strong that this CS is then capable of supporting operant behaviour independent of drug delivery, as demonstrated in subsequent training sessions in which the response requirements are progressively increased such that animals must respond numerous times to receive presentation.
of the CS, and numerous CSs prior to receipt of a single drug infusion. Such second-order schedules allow for the extensive study of the neurobiology underlying responding for drug in the absence of any confounding behavioural effects caused by drug delivery.

All three processes- ST, PIT, and CRf, can be considered somewhat hierarchically in that the property of cues which they measure increases in behavioural significance, from attracting interest (ST), to influencing ongoing goal-directed behaviour (PIT), and finally to becoming the goal itself (CRf). All of these cue-driven process have also been implicated in addiction, but in subtly different ways. As discussed above, ST is thought to reflect the degree to which cues paired with addictive drugs can induce the desire to use (Flagel, Akil, & Robinson, 2009). PIT taps into the process by which ongoing goal-directed behaviour can be influenced by encountering reward-paired cues, and thus may reflect how cue-induced craving translates into active drug-seeking (Tomie, Grimes, & Pohorecky, 2008). Evidence also suggests that the cues associated with drug-taking become CRfs, and represent autonomous sub-goals in their own right that are valued independently from the drug themselves. This powerful observation helps explain why drug substitution therapy can combat the physiological symptoms associated with drug withdrawal but does not necessarily reduce craving and the desire to use; the addict still yearns for the sensory experience triggered by the drug-paired cues (Naqvi, Rudrauf, Damasio, & Bechara, 2007). The degree to which individuals vary in their willingness to work for CRfs may therefore have a direct relationship to relapse vulnerability, particularly at time points distal to cessation of use, long after physiological withdrawal has passed. Interestingly, responding for CRfs is higher in rats during adolescence, a developmental period associated with higher vulnerability to addiction (Burton, Noble, & Fletcher, 2011).
While PIT, CRf and ST tasks all provide valuable information into the ways in which cues modulate behaviour, they are somewhat removed from the specific type of decision making that is recruited in the context of gambling and even relapse to addiction. Furthermore, although ST, PIT and CRf may look superficially quite similar, they depend on somewhat distinct neural and neurochemical systems that nevertheless overlap with those involved in addiction and affective decision-making within the limbic corticostriatal loop (Cardinal, Parkinson, Hall, & Everitt, 2002). Given that very similar-looking cue-dependent behaviours can depend on dissociable neurobiological substrates, the question then remains as to whether the influence of cues in more complex cognitive processes, such as the kinds of cost/benefit decision-making involved in gambling behaviour, is subject to similar or distinct regulatory mechanisms.

To this end, a modified version of the rGT that incorporates salient cues could provide insight into these questions. 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. The development of a cued version of the rGT would allow for the systematic investigation of cue-biased decision making and the neural circuitry that modulates the acquisition and expression of this behaviour.

1.4 Regional contributions to gambling-like behaviour
One of the principle benefits of the development of animal models of decision making (like those described above) is that researchers can employ techniques that would be ethically or technically impossible in human subjects. Chief among these are techniques such as pharmacological or chemogenetic inactivation that allow researchers to isolate the contributions of specific regions of the brain to the behaviours in question. Integrating these techniques with the aforementioned models of gambling-like behaviour could clarify the regions and circuitry that are recruited in
each task. A variety of subregions have been implicated in gambling and gambling-like behaviour and are thus attractive targets for investigation. Some of the most prominent of these are discussed below.

1.4.1 vmPFC

A seminal investigation into the contributions of the prefrontal cortex to decision making found that damage to the vmPFC (which encompassed the OFC, IL and PrL) in humans was associated with riskier, more disadvantageous choice behaviour relative to that of healthy controls (Bechara et al., 1994). Subsequent work using the same task suggested that the subregions implicated in the original publication made more nuanced contributions to behavior than had originally been described; damage to the IL and PrL was associated with this pattern of risky choice, whereas damage to the OFC instead appeared to impair reversal learning rather than promoting a general shift towards a riskier choice strategy (Fellows, 2004). Given these findings and the regions’ substantial projections to the nucleus accumbens, another region of central importance to motivated behaviour (Brog, Ongse, Deutch, & Zahm, 1993), the contributions of the orbitofrontal cortex (OFC), infralimbic cortex (IL) and prelimbic cortex (PrL) to gambling-like behaviour are of considerable interest.

The OFC has been implicated in a variety of functions related to affective decision making (Kringelbach & Radcliffe, 2005), and appears to be the primary site for “common currency computation”, whereby the various properties of a reward are integrated (Pearson, Watson, & Platt, 2014). The OFC tempers the impact of larger, uncertain rewards on behaviour in some contexts (Stopper, Green, & Floresco, 2014), but past work with the rGT suggests it is more important to the acquisition of optimal choice strategies than it is the execution of those choices on this task (Zeeb & Winstanley, 2011). In this work, lesions of the OFC made before
learning of the rGT delay the acquisition of an optimal choice strategy, but animals eventually come to perform at the same level as controls. Lesions made after animals had been trained on the task have no effect on performance (Zeeb & Winstanley, 2011).

However, the contributions of the OFC to reward-related behavior appear to be modulated by the presence of salient cues; work in our laboratory has demonstrated that the addition of a cue light that signalled choice of the larger delayed option on a delay discounting task increased animal’s choice of that option relative to an uncued version of the task. This effect seemed to be mediated by the OFC, as inactivation of the region decreased choice of the larger, cued option in the optimally performing rats. In contrast, OFC inhibition improved choice amongst the most impulsive animals on the uncued version of the task (Zeeb, Floresco, & Winstanley, 2010a). Therefore, the OFC may be recruited by the CrGT in a distinct way from that in which it is during performance of the rGT.

Together, the IL and PrL comprise the medial PFC, a region implicated in a wide variety of cognitive functions critical to decision making including (but not limited to) behavioural flexibility, encoding of action/outcome relationships, impulse control and behavioral inhibition (Orsini, Moorman, Young, Setlow, & Floresco, 2015). While often treated as a homologous unit, the IL and PrL have distinct patterns of afferent projections (Vertes, 2004) and appear make dissociable contributions to behavior (Peters, Kalivas, & Quirk, 2009). It has been proposed that the PrL generally serves to drive the execution of behaviour and the acquisition of reward, whereas the IL is generally to be important to the inhibition of behaviour (Orsini et al., 2015). While this is necessarily an oversimplification of the roles of each region, it provides a useful framework from which to consider the regions’ contributions to behaviour on our laboratory tasks of decision making. Work performed thus far has demonstrated that inhibition of both
regions taken together leads to a slight but significant increase in disadvantageous choice at baseline on the rGT (Zeeb, Baarendse, Vanderschuren, & Winstanley, 2015; Paine et al., 2013). The cognitive mechanisms by which IL/PL inhibition increases disadvantageous choice on the rGT are unclear, and replicating this manipulation on different but related tasks like the rBT may clarify the contributions of these regions to these forms of decision making.

1.4.2 BLA

The basolateral amygdala is a subdivision of the amygdala complex which receives input from sensory cortices, is reciprocally connected to the prefrontal cortex and projects to the striatum (Mcdonald, 1998). This pattern of connectivity makes it well-situated to contribute to motivated behaviour; early research into the behavioural role of the BLA demonstrated that lesions to the region impaired the ability of animals to learn to avoid stimuli that had been paired with electrical shocks (Weiskrantz, 1956) or otherwise respond appropriately to fearful stimuli (Blanchard et al., 1972). A substantial body of research has confirmed the importance of the BLA to performance on these so-called conditioned fear paradigms (Adolphs, Tranel, Damasio, & Damasio, 1995; Erlich, Bush, & Ledoux, 2012; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998), leading to the understanding that the BLA is essential to behaviour in the context of aversive stimuli. Lesions to the BLA shift animal’s preference away from small, unpunished rewards and towards options associated with larger rewards but also large punishments, (Orsini, Trotta, Bizon, & Setlow, 2015). Similarly, inactivation of the BLA promoted a punishment-resistant behavioural phenotype in rodents, where they were more likely than controls to respond for sucrose rewards when these were paired with the possibility of foot-shock punishments, indicating a dampened sensitivity to the motivational value of aversive stimuli (Piantadosi et al., 2017). Work with the rGT has shown that lesions of the BLA affect choice behaviour on the task
in distinct ways depending on when those lesions were made. Lesions made before the task has been learned delay the acquisition of an optimal strategy, whereas lesions made after the task has been learned lead to a more disadvantageous preference for the risky options (Zeeb & Winstanley, 2011). On the IGT, human subjects with amygdala damage showed lower skin conductance responses relative to healthy controls both in anticipation to and following risky decisions (Bechara et al., 1999). Taken as a whole, this research indicates that the BLA guides behaviour in the context of aversive or fear-inducing stimuli, and that the region integrates information about risk and punishment in the context of complex cost-benefit decision making paradigms.

However, the BLA is not simply a fear encoder. In contrast to early research which focused on aversive stimuli, more recent work has demonstrated that the BLA plays an integral role in reward related behaviour as well. Neurons in the BLA respond to reward-paired stimuli (Belova et al., 2008), and BLA firing both precedes and promotes activity in the NAc (Ambroggi, Ishikawa, Fields, & Nicola, 2008), which in turn guides motivated behaviour. In fact, distinct populations of neurons within the BLA may represent separate and competing pathways that encode both fear and reward (Janak & Tye, 2015). Other researchers have proposed that the BLA serves to integrate environmental stimuli with the current biological value of reinforcers, allowing individuals to make decisions on the basis of updated assessments of value rather than those that have been learned in other circumstances or under other conditions (Baxter & Murray, 2002). Granular work using optogenetic techniques to inhibit BLA activity at specific time points during the decision making process have added further complexity to the discussion of the region’s role in motivated responding. Inhibition of the BLA on a risky decision making task that took place while the animal was deliberating decreased choice of large
rewards that carried a risk of foot shock punishment (Orsini et al., 2017). However, BLA inhibition during the reward/punishment phase increased subsequent choice of risky options. The BLA appears to promote either risky or safe choice on this task, depending on the point at which activity takes place. In sum, the role of the BLA in risky decision making is complex, and the region appears to respond to stimuli of varying valence and salience. The addition of reward-paired cues to the rGT may meaningfully change the region’s contributions to reward-related decision making.

1.4.3 NAc

The NAc has sometimes been described as a “reward center”, given that neurons in the region track both reward-predictive cues and the delivery of reinforcing stimuli, and this excitation appears to be necessary for behavioral responding. (Ambroggi et al., 2008; Roitman, Wheeler, Wightman, & Carelli, 2008). However, this characterization is probably an oversimplification. The true function of the NAc appears to be considerably more complex than simply registering and responding to reward (Sesack & Grace, 2009). The NAc has extensive inputs from limbic, midbrain, prefrontal and temporal regions and projects to subcortical motor regions, making it perfectly positioned to take input from a variety of regions which are necessary for goal-directed behaviour and translate it into motivationally relevant motor output (Brog et al., 1993; Heimer, Louis, Anatomy, & Olmos, 1991; Sesack & Grace, 2009). For this reason, it is commonly referred to as the limbic-motor interface. Furthermore, the NAc appears to have greater influence over behaviour when the demands on behaviour are relatively more complex or under the influence of salient cues. Inactivation of subregions of the NAc does not prevent feeding behaviour, but in fact increases it (Reynolds & Berridge, 2002), and animals are able to learn and perform basic forms of instrumental responding without the benefit of a functional NAc.
In contrast, NAc inhibition prevents conditioned stimuli from invigorating behaviour; lesions of the NAc prohibit a conditioned stimulus from invigorating instrumental responding in PIT (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001), as well as preventing the acquisition of autoshaping behaviour in which a reward-predictive cue gains the ability to promote approach to that cue (Parkinson, Willoughby, Robbins, & Everitt, 2000). These and other findings support the idea that the NAc is critical to the ability of conditioned stimuli to enhance and influence motivated behaviour (Cardinal, Parkinson, Hall, & Everitt, 2003), making it a region of particular interest to us in our study of the motivational properties of salient cues.

The NAc is comprised of two primary subregions—the core (NAcC) and shell (NAcS), and these appear to make distinct contributions to behaviour. A reasonable summary of NAcC function is that is serves to guide motivated behavior towards desired options (Floresco, 2015). Excitation in the NAcC is precisely correlated with latency to approach a reward, suggesting that it plays a causal role in invigorating approach and engagement with reward and reward-related stimuli (du Hoffmann & Nicola, 2014). Additionally, NAcC inactivation increased the latency to make a response to a conditioned stimulus, reduced the rate of lever pressing for reward, and decreased checking for reward, collectively indicating that the NAcC is necessary to facilitate task engagement (Ambroggi, Ghazizadeh, Nicola, & Fields, 2011). This approach behaviour is likely modulated by DA release in the NAcC, which makes the region more sensitive to inputs from its various cortico-limbic afferents (Floresco, 2015).

In contrast, the NAcS appears to be important to inhibiting inappropriate behaviours that would otherwise hinder appropriate goal-directed action (Floresco, 2015). In one example, inactivation of the NAcS increased responding in a test of cue-induced reinstatement, indicating that the
region normally plays a role in suppressing behavioral responding to irrelevant or non-rewarded stimuli (Di Ciano, Robbins, & Everitt, 2008). Furthermore, inactivation of the NAcS increased responding to a cue that was explicitly not paired with reward, again suggesting that the shell plays a critical role in response inhibition to less relevant stimuli (Ambroggi et al., 2011). The NAcS receives extensive projections from cortical regions including the PrL, IL and OFC (Brog et al., 1993), and the ability of the NAcS to suppress behaviour that is irrelevant or otherwise inappropriate could conceivably be related to the higher-order input from these afferents.

1.5 Dopaminergic contributions to gambling behaviour

The neurotransmitter dopamine has attracted enormous interest for its potential role in addiction (see Koob, 1992; Volkow, Fowler, Wang, & Swanson, 2004 for review). Five subtypes of dopamine receptors have been identified and classed into two families. D₁-like receptors (D₁ and D₅) are excitatory and distributed widely throughout the cortex, striatum, NAc, amygdala and substantia nigra, while occurring to a lesser extent in other regions. D₂-like receptors (D₂, D₃, D₄) are inhibitory, and D₂/3 receptors are primarily expressed in the striatum and the nucleus accumbens, but also occur in limbic regions and the midbrain. D₄ receptors have a more limited range and are primarily distributed in areas of frontal cortex and limbic structures (Missale et al., 1998).

There is substantial evidence implicating DA in gambling behaviour. Researchers have demonstrated that amphetamine (a psychostimulant that increases dopamine presence in the synapse) 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). Methylphenidate, which prolongs the action of DA at the synapse by blocking reuptake, increases escalation of commitment in which individuals take risky bets in order to recover money that has already been lost, despite the low odds of recovery. This failure to inhibit risky choice could be due to an overestimation of the probability of recovery, or an over assessment of the value of the amount that could be recovered (Campbell-Meiklejohn et al., 2012). Iatrogenic GD can be induced in Parkinsonian patients being treated with dopamine agonists (Weintraub et al., 2006), and some evidence suggests that D2-like agonists with high affinity for D3 receptors such as pramipexol and ropinirole may be more likely than other classes of DA agonists to induce GD (Dodd et al., 2005). Along with evidence from genetic studies that report a relationship between D3 polymorphisms and elevated rates of GD as well as riskier gambling-like behaviour (Lobo et al., 2015; Lobo et al., 2010), it appears that D3 receptors are particularly important to gambling behaviour.

Despite the evident involvement of DA in disordered reward-related behaviour, choice patterns on the rGT are not predominantly driven by DA. Amphetamine-induced choice impairments are not mediated by the psychostimulant’s dopaminergic actions (Zeeb, Wong, & Winstanley, 2013). Furthermore, neither administration of D1-like or D2-like agonists nor a selective dopamine reuptake inhibitor affected choice (Baarendse & Vanderschuren, 2012; Zeeb et al., 2009). However, the presence of cues seems to recruit DA on a number of other tasks, and may also change DAs contributions to the rGT following the addition of salient cues. PIT can be enhanced by intra-NAC amphetamine and abolished by DA antagonists (Dickinson, Smith, & Mirenowicz, 2000), and ST is likewise sensitive to DAergic manipulations of the NAC (Dalley et al., 2005; Dalley et al., 2002). Administration of DA antagonists directly into the OFC decreased impulsive choice in a cued version (and not an uncued version) of the delay-
discounting task, theoretically by reducing the ability of the cue to promote choice of the larger delayed reward (Zeeb et al., 2010a). Clearly, the addition of cues can modify the influence of DA on behaviour, and it is thus possible that performance on a the CrGT will likewise be sensitive to DA manipulations.

1.5 Experimental objectives
The four experiments within this dissertation aim to clarify the cognitive mechanisms and neurobiological underpinnings of gambling-like decision making.
In experiment one, we added salient win associated audiovisual cues to the rGT to determine whether these would influence decision making, and if the addition of cues might change the pharmacology recruited by the task. Salient win related cues are an integral component of many human gambling paradigms, and their ability to disrupt this form of cost-benefit decision making has not, to the best of our knowledge, been examined. The effect of pharmacological manipulations targeting various DA receptor types was also examined. Although DA has been implicated in disordered gambling behaviour, rGT performance is relatively unaffected by DA manipulations; comparing the effect of DA manipulations in both a cued and uncued decision making task might clarify how DA is recruited by salient cues.

In experiment two, we evaluated the contributions of the basolateral amygdala (BLA) and orbitofrontal (OFC) cortex to decision-making on the cued rGT both during acquisition and once behaviour has been established. Prior work has demonstrated that lesions of both the OFC and BLA delayed learning of the uncued rGT, but only lesions of the BLA affected choice on the task once behaviour had been learned. Given that the addition of salient cues changes both baseline behaviour on the task and the contributions of DA to this behaviour, it seems possible that these cues might also change the manner in which the OFC and BLA are recruited by the cost-benefit decision-making process the task entails.
In experiment three, we examine the effects of chemogenetically inactivating either the NAcS or NAcC during both acquisition of the rGT and once baseline performance has been established. The NAc makes fundamental contributions to motivated responding, yet the region’s contributions to performance of the rGT have not yet been evaluated. The NAcC appears to be important for approach behaviour, while the NAcS may help gate the influence of irrelevant stimuli on behaviour; both of these processes may be recruited during rGT performance. This experiment should provide insight into the contributions of each subregion of the NAc to this particular form of cost-benefit decision making at different stages of learning.

In experiment four, animals were trained on the rBT before undergoing pharmacological inactivation of either the OFC, or the IL and PL cortices in order to determine the contributions of these regions to behaviour on the task. Performance on the rBT may be determined by an individual preference for either an objective choice strategy which weighs options according to their expected value, or a more subjective choice strategy that incorporates the size of the available reward available and the probability of reinforcement; inactivation of these regions will thus clarify their contributions to subjective choice biases.

Together, these avenues of research will meaningfully contribute to our understanding of the neurobiology and structural contributions to the type of decision making involved in gambling and substance addiction.
Chapter 2: General methods

2.1 Subjects
Subjects were male Long-Evans rats (Charles Rivers Laboratories, St. Constant, Quebec, Canada) weighing 250-300g upon 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 triple-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.

2.2 Behavioural apparatus
Testing took place in standard Med Associates 5-hole operant chambers housed in ventilated sound-attenuating cabinets (Med Associates Inc, Vermont, USA). Each chamber featured a food tray 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 this tray from an external food hopper. Two retractable levers were located at either side of this food magazine and a house light was positioned on the chamber wall above. 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 according to MedPC programs authored by CAW controlled by an IBM-compatible computer.

2.3 Behavioural testing
2.3.1 Habituation
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. Following these exposure sessions, animals underwent different training according to the task they would ultimately perform.

2.3.2 Training for the rat Gambling Task (rGT) and Cued rat Gambling Task (CrGT)

Animals who would perform the rGT or the CrGT were initially 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 then performed 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, and presence/absence of cues, were the same as those used in the full versions of the rGT (detailed below).

2.3.3 The rGT

A task schematic is provided in Figure 2-1. 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 in 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 (see Figure 2-1). 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 at the array 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 penalty time-out, after which the aperture light turned off, the tray light turned on, and the animal was able to initiate a new trial. If the rat responded in any aperture during the ITI, the trial was scored as a premature response, and the house light illuminated 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 begin a new trial.

Unlike tasks that utilize a block design (e.g. (Evenden & Ryan, 1999)), the reinforcement contingencies were kept constant throughout the session, and animals were free to choose from any option on every trial. Previous analyses indicate that choice patterns remain constant throughout the session (Zeeb et al., 2009). The different schedules of reward and punishment associated with each aperture resulted in unequal return across each 30 minute session. The optimal strategy was exclusive choice of P2, which would yield the maximal expected returns due to the relatively high probability of reward (0.8), and comparatively short (10s) and infrequent (p = 0.2) time-out penalties. 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. We have shown previously that these delay periods are critical in attenuating choice of the options associated with larger but less frequent rewards (Zeeb et al., 2009) The position of each option was counterbalanced across animals to mitigate any potential thigmotaxis-mediated biases towards the holes on the far side of the array. Version A (n=16) was arranged P1, P4, P2, P3 from left to right, and version B
(n=16) was arranged P4, P1, P3, P2. A total of 16 animals were tested on this version of the task, while an additional 16 were tested on the cued rGT.

2.3.4 The cued rGT

The structure of the cued rGT was identical to that of the original uncued rGT, save the introduction of audiovisual cues that accompanied reward delivery on winning trials and which varying in complexity across options. Comparable to the experience of human gambling games, the magnitude of win-associated cues became considerably larger as win size increased, as shown in Table 2-1. Prior to designing the cued rGT, we first used a simple flash frequency preference test to determine whether animals preferred slower vs faster frequencies of flashing light, and could discriminate between them. Each trial consisted of a choice between two flashing apertures, the location (holes one through five) and flash frequency (one to five Hz) of which was determined at random. A nose-poke in either of the illuminated apertures was always rewarded with delivery of a sugar pellet at the food tray. Animals (n = 16) showed a clear preference for cue lights flashing at higher frequencies (Choice: $F_{4,56} = 12.71$, $p < 0.001$ data not shown). Choice of the three, four and five hertz options were significantly higher than choice of the one and two Hz options, hence we chose to use visual cues that flashed at a frequency of 5 Hz combined with a sequence of auditory tones that changed every 0.2s. Each reward-paired cue was concurrent with pellet delivery and lasted for two seconds in total, after which a new trial could be initiated. 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 1-second tones delivered in the same order on each trial. The cues associated with the larger rewards were more complex and
variable, in keeping with observations that rodents find such cues appetitive (Olsen & Winder, 2009). The six tones used were: 4,8,10,12,15,20 kHz. We have successfully used these tones as discriminative stimuli in other behavioral procedures (Rogers, Wong, McKinnon, & Winstanley, 2013; Winstanley, Cocker, & Rogers, 2011). Using the letters A-F to represent a different tone, the patterns for P3 were as follows: CDEDCDEDCD; CECEDEDECE. Similarly, the patterns for P4 were: ABCDEFEDCB; BCDCDEDEFE; CEDFCEBDAC; FEDCBAFEDC. With respect to the visual cues, the first light to flash was the hole associated with that response. For P3 and P4, the visual stimuli then became more varied in the last second of the cue, using sequences of multiple lights that change in sync with the tones. Lights could be illuminated together (as indicated by numbers in brackets) or independently. The following numbers correspond to the aperture, numbered from left to right of the operant box. The patterns for P3 were as follows: 5434543454; (5+3)4(5+3)4(5+3)4(5+3)4(5+3)4. The patterns for P4 were as follows: 1234543212; (2+4)(1+3+5)(2+4)(1+3+5)(2+4)(1+3+5)(2+4) (1+3+5) (2+4) (1+3+5); 1324354231; 3(2+4)(1+5)(2+4)3(2+4)(1+5)(2+4)3(2+4). The tone/light pattern played on each winning trial was determined randomly, but no pattern was presented on sequential trials. The traylight also flashed at a frequency of 5Hz in conjunction with the array lights and tones.

2.4 Surgery

Animals were anesthetized with 2% isoflurane in oxygen and then secured in a stereotaxic frame with the incisor bar set to -3.3. Once anesthetized, animals were given anafen and bupivacaine subcutaneously. Bore holes made in the skull according to the relevant stereotaxic coordinates. Anterior-posterior co-ordinates were calculated from bregma, and dorsal-ventral from dura.
For cannulations, bilateral 22-gauge stainless-steel guide cannula (Plastics One, Roanoke, Virginia, USA) were inserted through the bore holes and then secured with bone screws and dental cement. Obdurators (flush with guides) affixed to plastic dust caps (Plastics One, Roanoke, Virginia, USA) were inserted into the cannulae to protect the infusion site. For viral infusions, 29-gauge injectors were lowered into the bore holes and animals were infused with 0.5 µl solution via a dual-channel infusion pump (Harvard Apparatus, Holliston, MA, USA) at a rate of 0.25 µl per minute. Injectors were left in place for ten minutes following the infusion to allow the solution to diffuse. Animals were given a week to recover in their home cage before behavioural testing resumed.

2.5 Infusions

The infusion procedure was based on previously published methods (Winstanley et al., 2003). During the infusion, animals were gently restrained while their obdurators were removed and 29-gauge injectors were inserted into the guides. Once these were inserted, animals were infused with 0.5 µl solution via a dual-channel infusion pump (Harvard Apparatus, Holliston, MA, USA) at a rate of 0.25 µl per minute. Injectors were left in place for one minute following the infusion to allow the solution to diffuse. Injectors were then removed, and autoclaved obdurators were once again affixed to the cannulae. Animals were returned to their home cages for a period of ten minutes to allow the drug to take effect, and then moved to the operant chamber to perform the task. Infusions took place in a procedure room adjacent to the operant behavioural testing room. Animals were given two mock infusions to habituate them to the infusion procedure.

2.6 Behavioural measurements

2.6.1 The rGT and CrGT

The primary dependent variable, choice of each individual option, was calculated as [(all
choices of a given option)/(total trials completed)]*100. Calculating choice preference as a percentage rather than as a raw count controlled for differences in total trials executed across sessions and between animals (Zeeb et al., 2009). 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: 

\[(\text{choice of P1})+(\text{choice of P2})-(\text{choice of P3})+(\text{choice of P4})\] (Zeeb & Winstanley, 2011). A positive score indicated the rat had adopted the optimal choice strategy favoring the advantageous options, whereas a negative score indicated a net preference for the high-risk, disadvantageous options.

As previously described, any response made during the ITI was scored as a premature response, and these were calculated as \([(\text{total premature responses})/(\text{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 as no main effect of session or choice x session interaction term when analyzing data from 3 consecutive days).

### 2.7 Data Analysis

All data analysis was performed with either SPSS for Mac (Version 22.0.0; IBM) or R (Version 3.4.4; GNU). 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.
<table>
<thead>
<tr>
<th>Option</th>
<th>Cue duration</th>
<th>Auditory cues</th>
<th>Visual cues</th>
<th>Variable?</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>2s</td>
<td>1 tone</td>
<td>Flash H1, 2.5Hz, 2s</td>
<td>No</td>
</tr>
<tr>
<td>P2</td>
<td>2s</td>
<td>2 tones, in sequence, 1s each</td>
<td>Flash H4, 2.5Hz, 2s</td>
<td>No</td>
</tr>
<tr>
<td>P3</td>
<td>2s</td>
<td>3 tones, in sequence, 0.2s each</td>
<td>Flash H5, 5Hz, 1s, Flash H2, H3, H4, 5Hz, 1s</td>
<td>Yes- 2 patterns</td>
</tr>
<tr>
<td>P4</td>
<td>2s</td>
<td>6 tones, in sequence, 0.2s each</td>
<td>Flash H2, 5Hz, 1s, Flash H1, H2, H3, H4, H5, 5Hz, 1s</td>
<td>Yes- 4 patterns</td>
</tr>
</tbody>
</table>

Table 2-1: Details of cues used for different reward sizes in the cued rGT
Figure 2-1: Task schematic of cued and uncued rGT paradigm. The rGT began with illumination of the traylight. A nosepoke response in the food tray extinguished the traylight and initiated a new trial. After an inter-trial-interval (ITI) of 5 sec, four stimulus lights were turned on in holes 1, 2, 4 and 5, each of which was associated with a different number of sugar pellets (P1-P4). The animal was required to respond in one of these holes within 10 sec. This response was then rewarded or punished depending on the reinforcement schedule for that option (indicated by the probability of a win or loss in brackets). If the animal was rewarded in the uncued task, the stimulus lights were simply extinguished and the animal received the corresponding number of pellets in the now-illuminated food tray. In the cued task, reward delivery was also accompanied by audiovisual cues that increased in complexity with reward magnitude (see Table 2-1 for details). The duration of the cues was held constant at 2s across all options. In both task variants, a response at the food tray then started a new trial. If the animal was punished, the stimulus light
in the corresponding hole flashed at a frequency of 0.5Hz for the duration of the punishing timeout, and all other lights were extinguished. At the end of the punishment period, the tray light was turned on and the animal could initiate a new trial. Failure to respond at the illuminated holes resulted in an omission, whereas a response during the ITI was classified as a premature response and punished by a 5 sec time out during which the houselight was turned on. The order of the options from left to right is counter-balanced within each cohort (version A as shown, version B: 4,1,3,2) The maximum number of pellets that could be theoretically obtained if the option was chosen exclusively in a 30 min session (not allowing time for choice/food consumption for consistency) is given, hence providing an objective value for each option.
Chapter 3: Dopamine D3 receptors modulate the ability of win-paired cues to increase risky choice in a rat Gambling Task

3.1 Introduction

Gambling disorder (GD), in which individuals lose control over their gambling behavior, leads to severe personal, social and financial consequences. Estimates suggest around 2.5% of the general population meet the criteria for GD, with a further 4.9% exhibiting troubling yet preclinical symptomatology (Cunningham-Williams et al., 2005; Shaffer, Hall, & Vander Bilt, 1999). A better understanding of the neuropathology underlying GD would be helpful in developing effective therapeutic interventions (Madden, Ewan, & Lagorio, 2007).

To this end, several behavioural paradigms have been designed to evaluate gambling-like behaviours in laboratory animals. The rat gambling task (rGT; (Zeeb et al., 2009)) is loosely based on the Iowa Gambling Task used clinically to assess decision-making under uncertainty. In both tasks, subjects choose between four options, each resulting in distinct patterns of gains or losses according to probabilistic schedules, with the goal of accruing reward. The best strategy is to favor options associated with smaller gains but also smaller penalties, and avoid the tempting “high-risk high-reward” outcomes; although such options can yield greater rewards per trial, the disproportionately larger punishments result in considerably less benefit over time.

Choice on the rGT and IGT is mediated by similar neural circuitry involving the medial prefrontal cortex, orbitofrontal cortex and basolateral amygdala (Bechara et al., 1999; Fellows, 2004; Paine et al., 2013; Zeeb & Winstanley, 2011; Zeeb & Winstanley, 2013). Surprisingly, in spite of substantial evidence implicating dopamine in reward-related behaviour (Bergh, Eklund, Södersten, & Nordin, 1997; Shinohara et al., 1999; Zack & Poulos, 2004) and in the iatrogenic development of GD in Parkinsonian patients (Weintraub et al., 2006), choice patterns on the rGT are not predominantly driven by the dopamine system. Amphetamine-induced choice
impairments are not mediated by the psychostimulant’s dopaminergic actions (Zeeb et al., 2013). Furthermore, neither administration of D_{1}-like or D_{2}-like agonists, nor a selective dopamine reuptake inhibitor, affected choice (Baarendse & Vanderschuren, 2012; Zeeb et al., 2009).

Highly salient win-associated cues are a significant component of human gambling, yet these are notably absent in the rGT. While cues predictive of reward increase the release of dopamine (Schultz, 1998), dopaminergic neurons appear to fire more strongly when reward delivery is probabilistic, with the greatest increase occurring when uncertainty is maximised (Fiorillo, 2003). Mice will also work for presentation of complex, variable audiovisual cues in a dopamine-dependent manner somewhat similar to cocaine self-administration (Olsen & Winder, 2009). Sensitivity to the behavioural influence mediated by reward-paired cues has long been associated with vulnerability to drug addiction and the propensity to relapse (Everitt et al., 2000, Kruzich et al., 2001; Saunders & Robinson, 2010), and may also mediate the transition from recreational to problem gambling (Grant & Bowling, 2014; van Holst et al., 2012). A robust demonstration of cue-induced maladaptive decision making would therefore be of value to the study of both gambling and substance use disorder, in addition to improving the construct validity of the rGT. We therefore hypothesized that adding win-related cues to the rGT, which increased in variety and complexity with reward size, would exacerbate risky decision-making and enhance the role of the dopamine system in mediating choice.

3.2 Additional methods

3.2.1 Subjects

Subjects were 48 male Long-Evans rats. Three groups of 16 rats were tested in series, one group on the uncued rGT, one group on the cued rGT, and one group on the cue preference task. One animal in the uncued group was excluded from all analyses due to unresolved behavioral instability.
3.2.2 The Cue Preference Task (CPT)

In order to control for the specific contributions of salient cues alone to choice behavior, irrespective of any conditioned associations between the cues and particular outcomes, an additional 16 animal cohort was trained on the Cue Preference Task (CPT). This task was identical to the cued rGT, except that a response to any of the four apertures was rewarded with a single sucrose pellet on an FR1 schedule, with no possibility of punishment. The win-related cues were identical to those on the cued rGT. For example, while selection of the P4-designated aperture on the CPT results only in one sucrose pellet, the delivery of this reward was still paired with the complex P4-associated win cues. This relationship is consistent across all options. As on both versions of the rGT, the location of each option was counterbalanced across the group. 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.

3.3 Results
3.3.1 Baseline choice behavior

Both groups trained on versions of the rGT reached behavioral stability at the same time point (Sessions 35-37; Session x Choice: F<sub>6,174</sub> = 0.39, p = 0.885; Session x Choice x Cue: F<sub>6,174</sub> = 1.03, p = 0.409). Animals performing the cued rGT demonstrated a significantly more disadvantageous choice preference as compared to those trained on the uncued rGT (Figure 3-1A; Choice x Group: F<sub>3,87</sub> = 4.12, p = 0.009). On the basis of this group difference, we examined choice by choice differences between cohorts trained on each task. On average, rats performing the cued task exhibited a reduced preference for P2, the best option, and also chose P3, one of the disadvantageous options, more frequently (Group- P2: F<sub>1,29</sub> = 6.44, p = 0.017, - P3: F<sub>1,29</sub> = 7.89, p = 0.009). No other behavioral measures differed significantly between groups (All F ≤ 3.32, all p ≤ 0.079). All animals trained on the CPT reached behavioral stability at the same
time point (Sessions 3-5; Session x Choice: F<sub>6,90</sub> = 1.31, p = 0.258). In contrast to the rGT and the cued rGT, animals performing the CPT did not demonstrate a significant preference for any option and sampled fairly equally between all 4 holes, although choice of the option associated with the most complex cue was greatest (Figure 3-1B; Choice: F<sub>3,45</sub> = 1.49, p = 0.231).

There were no changes in choice behavior once animals achieved stability. Behavior at baseline was compared to behavior during the vehicle injection day for each drug; no significant differences in choice behavior were found (vehicle: all F values ≤ 3.08, all p values ≥ 0.101; vehicle x choice: all F values ≤ 1.99, all p values ≥ 0.12). Additional behavioral measures are provided in Table 3-3.

### 3.3.2 Amphetamine

In keeping with previous reports, amphetamine increased choice of P1 and decreased choice of P2 in the uncued group (Figure 3-2; Dose x Choice: F<sub>9,126</sub> = 5.58, p < 0.001, sal vs 0.3 mg/kg: F<sub>3,42</sub> = 5.44, p =0.003, sal vs 1.0 mg/kg: F<sub>3,42</sub> = 8.39, p < 0.001, sal vs 1.5 mg/kg: F<sub>3,42</sub> = 10.76, p < 0.001), while there was no significant effect of drug on choice behavior in the cued group (Figure 3-2, Dose x Choice: F<sub>9,135</sub> = 1.177, p = 0.314).

The highest dose of amphetamine increased choice latency in the cued group, and while there was an overall significant effect of dose in the uncued group, no specific dose had a significant effects on this measure as compared to vehicle (Dose -Uncued: F<sub>3,42</sub> = 3.38, p = 0.027; -Cued: F<sub>3,45</sub> = 3.84, p = 0.016; sal vs 1.5mg/kg: Cued F<sub>3,45</sub> = 3.84, p = 0.016, 1.23 +/- 0.20 vs 1.67 +/- 0.25). A robust increase in premature responding was observed in both cohorts (Dose-Uncued F<sub>3,42</sub> = 2.39, p = 0.049; -Cued: F<sub>3,87</sub> = 7.96, p < 0.001; sal vs 0.3 mg/kg- Uncued F<sub>3,42</sub> = 2.39, p = 0.049, 27.19 +/- 4.02 vs 41.34 +/- 3.83; -Cued F<sub>3,45</sub> = 6.44, p = 0.001, 25.94 +/- 4.31 vs 44.81 +/- 4.61), and omissions were increased by amphetamine in the cued group (Dose- Uncued F<sub>3,42</sub> = 1.34, p = 0.275; -Cued: F<sub>3,45</sub> = 3.44, p = 0.025, sal vs 1.5mg/kg: F<sub>3,45</sub> = 3.84, p = 0.016,
0.75 +/- 0.23 vs 5.13 +/- 2.23). No other variable was affected by the drug (all Fs < 2.81, NS).

3.3.3 Eticlopride

In contrast to previous reports (Zeeb et al., 2009; Zeeb et al., 2012), the D2 antagonist eticlopride did not improve performance by increasing choice of the best option in the uncued procedure, and a similar null effect on choice behavior was observed in the cued version of the task (Dose- Uncued F3,45 = 1.10, p = 0.358; -Cued F3,39 = 1.23, p = 0.310; Dose x Choice - Uncued; F9,135 = 0.89, p = 0.529; -Cued: F9,117 = 1.07, p = 0.391).

Eticlopride increased the latency to make a choice (Dose- Uncued: F3,45 = 6.02, p = 0.002; Cued: F3,39 = 7.75, p < 0.001; sal vs 0.06 mg/kg: -Uncued F1,15 = 10.04, p = 0.006, 0.94 +/- 0.08 vs. 1.48 +/- 0.16; -Cued: F1,14 = 15.36, p = 0.002, 0.96 +/- 0.14 vs 1.53 +/- 0.19), and decreased premature responses (Dose: -Uncued F3,45 = 5.54, p = 0.003; - Cued: Dose: F3,39 = 7.725, p < 0.001; sal vs 0.06 mg/kg- Uncued: F1,15 = 8.49, p = 0.011, 23.36 +/- 3.23 vs 13.89 +/- 2.52; - Cued: F1,14 = 13.48, p = 0.003; 27.48 +/- 3.90 vs. 14.71 +/- 2.56). Eticlopride also slightly increased omissions in the uncued group (Dose: -Uncued: F3,45 = 3.90, p = 0.015; saline vs 0.06 mg/kg: F1,15 = 4.82, p = 0.044; 0.27 +/- 0.15 vs 2.60 +/- 1.05; - Cued: F3,39 = 0.92, p = 0.438), collectively indicative of motor slowing at higher doses as expected following higher doses of dopamine antagonist administration. There were no effects on any other behavioral measure (All Fs ≤ 1.44, p ≥ 0.243).

3.3.4 PD128907

rGT and cued rGT

The highest dose of the D3 agonist PD128907 increased choice of the risky, disadvantageous option P3, but only in the cued group (Figure 3-3A, 4B, Table 3-2; Dose-
Uncued: $F_{3,21} = 1.94, p = 0.154$; -Cued: $F_{3,21} = 3.32, p = 0.039$; saline vs 5mg/kg: dose: $F_{1,7} = 6.628, p = 0.04$.

While PD128907 had no effect on premature responses in the cued group, the lowest and highest dose increased and decreased this form of impulsivity respectively in the uncued group (Dose- Uncued: $F_{3,21} = 8.58, p = 0.001$; sal vs 0.01 mg/kg- $F_{1,7} = 9.05, p = 0.020$, mean ± SEM: 14.36 ± 3.23 vs 22.63 ± 4.51; sal vs 0.1 mg/kg- $F_{1,7} = 11.36, p = 0.012$, mean ± SEM: 14.36 ± 3.23 vs 10.56 ± 4.27; Cued: $F_{3,21} = 0.95, p = 0.43$). Choice latency, collection latency and omissions were also increased in the uncued group but not the cued group, though no individual dose was significantly different from saline (Dose: -Choice latency, -Uncued: $F_{3,21} = 3.59, p = 0.031$, mean ± SEM: saline: 0.95 ± 0.21, 0.01 mg/kg: 0.94 ± 0.23, 0.03 mg/kg: 0.98 ± 0.19, 0.1 mg/kg: 1.13 ± 0.10; -Cued: $F_{3,21} = 0.79, p = 0.512$; Collection latency, -Uncued: $F_{3,21} = 3.09, p = 0.049$, mean ± SEM: saline: 1.00 ± 0.10, 0.01 mg/kg: 0.99 ± 0.12, 0.03 mg/kg: 1.01 ± 0.13, 0.1 mg/kg: 1.11 ± 0.17; -Cued: $F_{3,21} = 0.77, p = 0.525$; Omissions, -Uncued: $F_{3,21} = 3.27, p = 0.041$; mean ± SEM: saline: 0.00 ± 0.00, 0.01 mg/kg: 0.25 ± 0.25, 0.03 mg/kg: 0.5 ± 0.38, 0.1 mg/kg: 0.71 ± 0.18; -Cued: $F_{3,21} = 1.93, p = 0.156$). Trials completed were not affected by the drug (Uncued: $F_{3,21} = 0.59, p = 0.63$; Cued: $F_{3,21} = 0.93, p = 0.443$).

**CPT**

PD128907 did not affect choice behavior in the CPT (Figure 3-3C, Table 3-2; Dose: $F_{3,42} = 1.93, p = 0.14$; dose x choice: $F_{9,126} = 0.00, p = 0.96$), and again did not exhibit a significant preference for any choice option (Choice: $F_{3,42} = 0.513, p = 0.675$). Similar to results from the uncued rGT, the highest dose decreased premature responses (Dose: $F_{3,45} = 6.74; p = 0.001$; saline vs 0.1mg/kg: $F_{3,45} = 15.78, p = 0.001$, mean ± SEM: 6.62 ± 1.85 vs 1.32 ± 0.43). Choice
latency also increased (Dose: $F_{3,42} = 4.23$, $p = 0.01$), again, an effect driven by a decrease at the highest dose (saline vs 0.1mg/kg: $F_{1,14} = 5.52$, $p = 0.034$, mean ± SEM: 2.26 ± 0.30 vs 2.93 ± 0.31). No other behavioral measures were affected (All $F$s ≤ 2.79, all $p$ ≥ 0.072).

3.3.5 SB277011-A

**rGT and cued rGT**

The lower doses of the D$_3$ antagonist SB277011-A had the inverse pattern of effects to PD128907 in the cued group, decreasing choice of the disadvantageous option P3, yet was without effect in the uncued group (Figure 3-3D,4E, Table 3-2; Dose: -Uncued: $F_{3,18} = 0.08$, $p = 0.969$; -Cued: $F_{3,21} = 3.07$, $p = 0.05$; saline vs 0.5mg/kg: $F_{1,7} = 6.78$, $p = 0.035$; saline vs 1.5 mg/kg: dose x choice $F_{1,7} = 4.81$, $p = 0.01$). The drug did not affect any other behavioral measures (All $F$ ≤ 2.73, $p$ ≥ 0.070).

**CPT**

SB277011-A did not affect choice behavior in the CPT (Figure 3-3F, Table 3-2; Dose: $F_{3,45} = 0.94$, $p = 0.428$; dose x choice: $F_{9,135} = 1.34$, $p = 0.224$), and animals did not exhibit a significant preference for any option (Choice: $F_{3,45} = 0.852$, $p = 0.473$). No other behavioral measures were affected (All $F$ ≤ 3.84, All $p$ ≥ 0.088).

3.3.6 A-381393

A-381393, a selective D$_4$ receptor antagonist, did not significantly affect choice in either group (Dose: Uncued: $F_{3,21} = 0.254$, $p = 0.254$; Cued: $F_{3,21} = 0.312$, $p = 0.817$; Dose x Choice: Uncued: $F_{9,63} = 1.22$, $p = 0.299$; Cued: $F_{9,63} = 1.88$, $p = 0.072$) or any other behavioral measures (All $F$ ≤ 2.69, All $p$ ≥ 0.060).
3.3.7 PD-168077

The D₄ receptor agonist PD-168077 did not affect choice behavior in either the cued or uncued groups (Dose: Uncued: F₃,₂₁ = 0.256, p = 0.856; Cued: F₃,₂₁ = 0.15, p = 0.929; Dose x Choice: Uncued: F₉,₆₃ = 0.56, p = 0.828; Cued: F₉,₆₃ = 0.44, p = 0.911). All other behavioral measures were likewise unaffected (All F ≤ 2.03, p ≥ 0.092).

3.4 Discussion

This work provides the first clear demonstration in an animal model that salient, audiovisual win-related cues are sufficient to enhance choice of riskier, more disadvantageous options, thereby modeling the negative impact such cues may have on human choice. Furthermore, the presence of such cues alters the way in which certain dopaminergic ligands impact decision-making. Choice on the cued task appears uniquely sensitive to modulation by D₃ receptor drugs; the agonist PD128907 increased choice of one of the high-risk options, whereas the D₃ antagonist SB277011-A had the opposite effect. These compounds did not affect choice in the uncued procedure nor the CPT, suggesting that cue-biased risky choice can be pharmacologically dissociated from both the process of discriminating between options associated with probabilistic reinforcement schedules, and from simply responding for cue-paired rewards. In contrast, amphetamine only drove a risk-averse shift away from P₂ and towards P₁ in the uncued task. Numerous studies specifically implicate D₃ receptors in mediating the maladaptive influence of cues in substance use disorder, and recent data posit a critical role for this receptor subtype in GD (Boileau et al., 2014; Lobo et al., 2015). The cued rGT may therefore provide a novel method to empirically determine the degree to which cue-sensitivity can promote poor choice in a cost/benefit model in a manner central to the addiction process.
Comparable null effects were observed across both task versions following administration of eticlopride. Previous publications either likewise report no effect of D_2-like antagonists on-task (Paine et al., 2013), or observed a small increase in preference for the most optimal P2 choice (Zeeb et al., 2013; Zeeb et al., 2009). The reason for these discrepant results are unclear, but collectively indicate that D_2 receptor blockade does not have robust effects on choice behavior. The selective D_4 agonist and antagonist were also equally ineffective at modulating performance of either rGT version. Although D_4 agents have not typically resulted in significant behavioral effects on a variety of cognitive procedures (Oak et al., 2000, Le Foll et al., 2009), this receptor subtype has been implicated in some aspects of addiction (Di Ciano, Grandy, & Le Foll, 2014), and in the attribution of incentive salience to subthreshold environmental stimuli during fear-conditioning (Lauzon & Laviolette, 2010). We also found that D_4 receptor ligands modulated the erroneous expectation of reward on a rat slot machine task (rSMT), in which the animal must correctly interpret a series of cue lights as being indicative of a win or loss in order to optimize reward earned (Cocker, Le Foll, Rogers, & Winstanley, 2014). In the rSMT, the cues are present during the selection and initiation of the operant response and, as per fear conditioning procedures, the cues are truly predictive of an outcome. In contrast, on the rGT, the cues are instead delivered after the choice has been made, and only when the outcome involves delivery of reward. Cue presentation is therefore reward-concurrent, rather than reward-predictive, and may therefore influence choice via an alternative mechanism.

Given our interest in the role of DA in performance of the CrGT, it may appear surprising that we did not include D_1-family drugs in the present study. However, work with the rGT and other tasks in our laboratory provides little evidence for D_1 receptor influence on this form of decision making. Zeeb et al. (2009) found that neither D_1 agonism nor antagonism (SKF 81297
and SCH 23390, respectively) affected choice on the rGT, though both drugs modified choice behaviour on simpler versions of the task, leading those authors to conclude that the specific form of risky decision making examined by the rGT in which multiple factors must be integrated was perhaps less D₁-dependent than decision making in which relatively fewer factors had to be considered. Furthermore, work with a rodent slot machine task (rSMT) found similarly null effects of the same D₁ agonist and antagonist on performance of that model of gambling behaviour (Winstanley et al., 2011). While the decision making processes underlying the rGT, CrGT and rSMT are not interchangeable, they each examine similarly complex models of gambling-like choice and presumably recruit at least somewhat similar neurobiology. This pattern of null results with D₁-family receptor compounds on gambling-like tasks thus lead us to omit them from the present experiment.

It is worth considering whether the cues inhibited learning, rather than biased “informed” choice, perhaps by confusing or distracting the animal. However, animals trained on both the cued and uncued versions of the task developed stable choice preferences within the same time frame. Similar to behavior on the uncued task, animals performing the cued rGT exhibited clear preference for one of the four options, indicating behavior is unlikely to be driven by random sampling. It thus appears that the cues neither enhanced nor impaired acquisition of the task, but simply drove preference for riskier outcomes. Animals on both tasks also made comparable numbers of premature responses and omissions, and latencies to choose an option and collect any resulting reward did not differ across versions. It is therefore difficult to attribute the increase in risky choice on the cued task to general changes in motivation, task engagement or a lack of awareness of the reward contingencies in play. As shown previously, choice could also be modulated independently from other behavioral variables, suggesting somewhat dissociable
pharmacological regulation of these distinct aspects of performance (Silveira, Malcolm, Shoaib, & Winstanley, 2015; Zeeb et al., 2009).

The question then remains as to the cognitive and neurobiological mechanisms by which reward-paired cues elicit such a shift in choice behavior. Given the numerous reports demonstrating that amphetamine potentiates the behavioral influence of reward-paired cues, the fact that amphetamine does not potentiates cue-induced risky choice appears to be something of an anomaly. For example, amphetamine increases responding for reward-paired cues in tests of conditioned reinforcement (Hill, 1970; Robbins, 1978), enhances Pavlovian approach to reward paired cues in sign-tracking procedures (Hitchcottet et al., 1997; Phillips et al., 2003), potentiates cue-induced relapse to drug-seeking (Saunderset et al., 2013), and also enhances the influence of reward-paired cues in a delay-discounting task (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). Amphetamine also increases choice of larger-uncertain options in other rodent tasks (Cocker, Hosking, Benoit, & Winstanley, 2012; St Onge & Floresco, 2009). If win-paired cues were enhancing risky choice through their ability to act as traditional conditioned reinforcers, or Pavlovian incentive stimuli, one would expect amphetamine to potentiate this cue-induced shift in preference towards P3. In contrast, amphetamine increased choice of P1 and decreased choice of P2 in the uncued task, consistent with previous reports, yet was without significant effects on the cued procedure.

A key difference between the rGT and the other behavioral procedures listed above is that a failure to win is explicitly punished by a signaled time-out, heavily cued by a flashing stimulus light. We originally postulated (Zeeb et al., 2009) that amphetamine’s ability to potentiate the behavioral influence of cues associated with aversive events (Killcross et al., 1997) led rats to favor the option associated with the shortest and least frequent penalties, P1. The null effect of
amphetamine in the cued group may therefore arise because the drug-induced increase in the motivational salience of the win-paired cues competed with, and subsequently mitigated, the behavioral impact of the loss-related cues, such that they were no longer sufficient to shift preference towards P1. Although speculative, such a hypothesis remains open to empirical verification in future studies.

Work with human subjects points to several mechanisms of GD that may have explanatory value in understanding the mechanisms by which the CrGT’s cues are able to potentiate choice of the risky options. Ring et al., (2018) provided evidence that problem gamblers rate small probabilities of gains as more attractive than healthy controls, but that both groups are equally sensitive to losses. It is possible that cues could be amplifying the attractiveness of wins on the CrGT by disrupting subjects’ ability to accurately gauge the probability of reward, thus promoting a GD-like bias towards larger but less certain options. However, this is in direct contrast with work done by Genauck et al., (2017), who found that individuals with GD show lower loss aversion and lower behavioural sensitivity to loss than normal controls. Relating this to the CrGT, it seems possible that the cues on the task may influence risky decision making either via increasing the attractiveness of uncertain options, or conversely, by decreasing the impact of losses associated with those options. These theories cannot be validated through experimentation using the CrGT because loss and gain dimensions cannot be disentangled without modifying the task structure. Training subjects on a simplified task based on the CrGT that modifies either the loss or the gain dimension of the task could enhance our basic understanding of how these cues shift behaviour towards more disadvantageous options. Beyond the marked difference in baseline behavior, the most striking distinction between the cued and uncued task is the degree to which risky choice is modulated by
D₃ ligands in the former, but not the latter. There was no significant preference for any of the cues in the CPT, nor did D₃ ligands modulate choice on this simple task, suggesting that these compounds are not simply augmenting or diminishing any affective value ascribed to the cues themselves. Numerous studies have implicated D₃ receptor signaling in the behavioral manifestation of drug addiction across a wide range of abused substances. Recent syntheses of the current literature indicate that D₃ receptors may play a particular role in mediating the effect of drug-paired cues on behavior; not only are CPP and cue-induced reinstatement robustly attenuated by D₃ antagonists, but D₃-selective compounds have much greater effects on responding for drug under second order schedules of reinforcement and higher FR schedules, in which cues play a clearer role in supporting operant behavior, than on simpler FR1 or FR2 schedules (Le Foll et al., 2005; Beninger & Banasikowski, 2008). However, although there is a relative paucity of data from studies that used non-drug unconditioned stimuli, the consensus appears to be that D₃ agonists and antagonists have little-to-no effect on such responding. For example, SB-270110-A did not affect responding on a second-order schedule for sucrose reinforcement (Di Ciano, Underwood, Hagan, & Everitt, 2003). Although higher doses of a less-selective D₃ agonist increased responding for food-paired CRf, this dose was likely acting at D₂ receptors (Sutton, Rolfe, & Beninger, 2001). This might also explain the non-linear dose response effect observed here of SB277011-A on choice on the CrGT, where the low and medium doses decreased choice of P3 yet the largest dose did not. While lower doses of SB277011-A might act selectively on D₃ receptors to reduce choice of P3, higher doses may also act at D₂ receptors and offset these effects. Similar non-linear effects on reward related decision making have been observed with other D₃-targeted ligands that are known to act at D₂ receptors at higher doses (Beninger & Ranaldi, 1992).
Whereas the D₂ receptor is expressed fairly ubiquitously in sites innervated by dopamine, the D₃ receptor is concentrated within the nucleus accumbens (NAC), islands of Calleja, and limbic structures such as the hippocampus and amygdala (Bouthenet et al. 1991; Levesque et al. 1992). The NAC, lateral habenula and central and basolateral amygdala have been identified as key sites at which D₃ receptors modulate behavioral models of drug addiction, although whether the same neural circuitry is involved in the modulation of cue-driven risky choice by D₃ ligands remains to be determined (Le Foll et al. 2014). A history of prior cocaine self-administration can enhance behavioral reactivity to D₃ ligands (Blaylock et al., 2011). It has also been suggested that repeated experience of a CS that predicts reward with maximal uncertainty (50%), or responding for unpredictable reinforcement under variable rather than fixed ratio schedules, can sensitize dopamine release (Zack et al., 2014, Singer et al., 2012). Given that the cues facilitated choice of the option associated with maximal uncertainty on the rGT (P3, 50% chance of 3 sugar pellets), the effects of the D₃ receptor agents may reflect long-term alterations in the sensitivity of the DA system caused by repeated choice of options with the most uncertain outcome, rather than modulation of cue-related behavior per se. This tentative hypothesis appears to be supported by the null effects of D₃ manipulations on choice in the CPT. If so, D₃ agents should also modulate choice in rats exhibiting high levels of risky choice on the uncued rGT. Attempts to confirm this may be limited by the fact that so few animals prefer the risky options at baseline. However, in as much as we were able to determine within the cohorts tested here, the magnitude of the behavioral change caused by D₃ ligands did not track the strength of the preference for P3 in the cued or uncued groups.

In sum, these data demonstrate that the addition of reward-paired cues to a rodent model of gambling-related decision making substantially increases maladaptive, risky choice. The
presence of cues also enhanced the role D₃ receptor-mediated signaling played in regulating choice behavior. This receptor subclass has been strongly implicated in addiction, and D₃-selective agents rarely modulate behavior supported by standard nutritional reinforcers. The cued rGT may therefore be relatively unique in its ability to capture decision-making deficits representative of those seen in addiction disorders, and underpinned by similar neurobiological processes. As such, drugs that can improve decision-making on this task may have significant clinical benefit in remedying the disordered decision making central to the maintenance of the addicted state, and which remains one of the most problematic and intractable features of behavioral and chemical dependency.
<table>
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<tr>
<th>Drug</th>
<th>Drug type</th>
<th>Dose (mg/kg)</th>
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<td>Non-selective dopamine agonist</td>
<td>Vehicle, 0.3, 1.0, 1.5</td>
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<tr>
<td>Eticlopride</td>
<td>Dopamine D2 family antagonist</td>
<td>Vehicle, 0.01, 0.03, 0.06</td>
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<tr>
<td>PD128907</td>
<td>Dopamine D3 agonist</td>
<td>Vehicle, 0.01, 0.03, 0.1</td>
</tr>
<tr>
<td>SB-277011-A</td>
<td>Dopamine D3 antagonist</td>
<td>Vehicle, 0.5, 1.0, 5.0</td>
</tr>
<tr>
<td>PD-168077</td>
<td>Dopamine D4 agonist</td>
<td>Vehicle, 0.5, 1.0, 5.0</td>
</tr>
<tr>
<td>A-391383</td>
<td>Dopamine D4 antagonist</td>
<td>Vehicle, 0.5, 1.0, 5.0</td>
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Table 3-1: Details of drug doses used.
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<td>P2</td>
<td>P3</td>
<td>P4</td>
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<td>11.25 ± 2.75</td>
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<td>10.43 ± 4.64</td>
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<td>7.74 ± 2.05</td>
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<td>7.86 ± 3.12</td>
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<td>4.93 ± 1.46</td>
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<td>38.31 ± 13.98</td>
<td>21.30 ± 13.09</td>
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<td><strong>Cue Pref</strong></td>
<td>Baseline</td>
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Table 3-2: Choice across each task.
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<td>Saline</td>
<td>83.20 ± 8.47</td>
<td>27.19 ± 4.02</td>
<td>1.27 ± 0.24</td>
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<td>2.53 ± 0.32</td>
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<td>6.47 ± 1.31</td>
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**Table 3-3: Additional behavioural measures.**
Figure 3-1: Risky choice is higher at baseline on the cued vs uncued rGT, but there is no significant difference across choice of individual options on the CPT. Data shown are mean ± SEM. * denotes p< 0.05.
Amphetamine significantly affects choice on the uncued but not cued rGT.

Higher doses of amphetamine significantly increased choice of P1 and decreased choice of P2 in the uncued rGT (A; P1, Dose- F$_{3,42}$ = 10.51, p < 0.001; sal vs 0.3 mg/kg: F$_{1,14}$ = 8.52, p = 0.011; sal vs 1.0 mg/kg: F$_{1,14}$ = 20.02, p = 0.001 sal vs 1.5 mg/kg F$_{1,14}$ = 19.18, p < 0.001; P2: Dose- F$_{3,42}$ = 5.43, p = 0.003; sal vs 0.3 mg/kg: F$_{1,14}$ = 5.55, p = 0.034; sal vs 1.0 mg/kg; F$_{1,14}$ = 6.23, p = 0.026; sal vs 1.5 mg/kg F$_{1,14}$ = 11.08, p = 0.005) but these effects did not reach statistical significance in the cued rGT (B). Data are graphed as the mean change from vehicle ± SEM, in order to illustrate the effect of each drug across task independent of the difference in preference for each option between task variants and individual subjects. * denotes p< 0.05.
Figure 3.3: Selective modulation of choice on the cued rGT by $D_3$ receptor ligands. The $D_3$ agonist PD128907 significantly increased P3 in the cued group ($P3, F_{3,21} = 3.32, p = 0.039$), but not in the uncued group. SB277011-A likewise selectively affected the cued group alone, and had the opposite pattern of effects to the agonist, decreasing choice of P3 ($P3, Dose: F_{3,21} = 4.41, p = 0.015$). Data are graphed as the mean change from vehicle ± SEM, in order to illustrate the effect of each drug across task independent of the difference in preference for each option between task variants and individual subjects.
Chapter 4: Inactivation of the orbitofrontal cortex, but not the basolateral amygdala, prevents the development of risky, cue-biased decision making on a rat Gambling Task

4.1 Introduction
Salient, audiovisual cues are a significant component of real-world gambling, and delineating the specific contributions of cues to risky decision making seems essential to understanding certain maladaptive behaviours that are affected by commercial gambling products, such as Gambling Disorder (GD) and co-morbid substance addiction (Barrus, Cherkasova, & Winstanley, 2016). Our lab has developed the Cued rat Gambling Task (CrGT), a derivative of the rat Gambling task (rGT) in order to study the interplay between salient, win-related audiovisual cues and optimal cost-benefit decision making (Barrus & Winstanley, 2016). Both the rGT and CrGT offer subjects a choice between options associated with smaller rewards and a lower probability of punishment, versus large rewards paired with a higher risk of punishment. On the CrGT, the delivery of small, certain rewards is accompanied by subtle audiovisual cues, whereas larger wins are accompanied by disproportionately more prominent cues. Work with the CrGT has demonstrated that the addition of these cues is sufficient to shift choice towards risky options, and that choice behavior on cued and uncued tasks is differentially sensitive to various pharmacological manipulations (Barrus & Winstanley, 2016), (Adams, Barkus, Ferland, Sharp, & Winstanley, 2017). However, we do not yet know if cues change the manner in which distinct regions of the brain are recruited into the cost-benefit decision-making process.

Previous research has demonstrated dissociable roles for the basolateral amygdala and the orbitofrontal cortex on the uncued version of the task (Zeeb & Winstanley, 2011; Zeeb & Winstanley, 2013). On the rGT, the OFC appears to facilitate acquisition of the most optimal choice strategy, yet ceases to be important for maintaining such a strategy once it has been established (Zeeb & Winstanley, 2011). Specifically, lesions of the OFC made before learning of
the rGT increased the number of sessions required before a clear preference developed for P2, but animals eventually come to perform at the same level as controls. Lesions made after animals had been trained on the task had no effect on performance (Zeeb & Winstanley, 2011). Lesions to the BLA delayed the acquisition of the optimal choice strategy, producing an identical pattern of effects to OFC lesions, but in contrast, lesions made after the task had been learned lead to increases in risky choice. These findings are in line with other work that has shown that damage to the BLA leads to reckless and risky behaviour (Bechara et al., 1999).

It is possible that the addition of cues may change the involvement of the BLA and OFC in this form of probabilistic decision-making. Neurons in the OFC appears to track the subjective value of stimuli in humans and animals (Kringelbach, O'Doherty, Rolls, & Andrews, 2003; Schultz et al., 1999), and encode both the motivational value and the salience of stimuli (Rothkirch, Schmack, Schlagenhauf, & Sterzer, 2012). The region has been proposed to represent a nexus for the integration of hedonic experience and external reinforcement (Kringelbach & Radcliffe, 2005). Work with rodent paradigms provides evidence to suggest that the OFC may promote subjective choice biases over more rational choice strategies; pharmacological inactivation of the OFC ameliorated a mathematically irrational aversion to uncertainty as measured by the rodent Betting Task (rBT) (Barrus, Hosking, Cocker, & Winstanley, 2016). Furthermore, work with a delay discounting paradigm, in which subjects choose between smaller-sooner versus larger-later rewards, found that the effects of OFC inactivation were dependent on the presence or absence of cues that bridged the delay. As such, the role of the OFC in decision making is modulated not just by economic components, but also by environmental stimuli signalling choice outcome (Zeeb, Floresco, & Winstanley, 2010b). Given that choice on the CrGT is heavily biased by the presence of these cues, the OFC may
play a pivotal role in promoting this choice bias. The BLA is another area of interest in the context of CrGT given its apparent role in integrating salient stimuli and decision making. In fear conditioning paradigms in which a salient cue is paired with an aversive stimuli such as a foot shock and animals develop a conditioned aversion to the cue, inhibition of the BLA leads to a decrease in this conditioned behavior (Phillips et al., 1992; Fanselow et al., 1994). Human patients with amygdala damage also appear to be insensitive to the consequences of risky decisions; they do not show the skin conductance response that precedes or follows a risky decision on the IGT in the manner that normal controls do (Bechara et al., 1999). More recent research suggests a role for the BLA in signalling positively valenced stimuli as well. The BLA shows activity in response to reward-paired cues, and this activity precedes and promotes firing in the NAc, enabling motivated responding (Ambroggi et al., 2008). Given this responsiveness to both positively and negatively valenced stimuli, the contributions of the BLA to performance the saliently cued CrGT are difficult to predict.

Inactivating the OFC and the BLA both while the animal is learning the task and then later once the task has been learned will clarify the period during which they contribute to decision making on the CrGT. Given the findings on the rGT, we hypothesize that OFC inactivation might ameliorate the influence of cues on choice on the task, thereby promoting a more optimal pattern of choice. BLA inactivations may elevate risky choice by impairing the region’s aforementioned ability to suppress choice of risky but disadvantageous options. Following these procedures, animals will be tested on a reward-devaluation paradigm to clarify whether behaviour on the CrGT is goal-directed.

4.2 Additional Methods

4.2.1 Subjects
Subjects were 80 male Long-Evans rats (Charles Rivers Laboratories, St. Constant, Quebec, Canada) weighing 250-275g at the time of arrival at the animal facility.

4.2.2 Surgery

Animals were split into two cohorts, termed either ‘acquisition’ (n = 48) or ‘baseline’ (n = 32) groups, which dictated the point at which animals underwent surgery and the subsequent infusions. Animals in the acquisition cohort underwent surgery after being habituated to the animal facility, but before any behavioural training. Animals in the baseline cohort underwent surgery after they had been trained to behavioral stability on the CrGT.

Animals received bilateral cannulations of either the basolateral amygdala (BLA) (n = 40) or the lateral orbitofrontal cortex (IOFC) (n = 40). Stereotaxic coordinates for the BLA were AP -3.0 (from bregma), ML +/-4.8, DV -7.8. Stereotaxic coordinates for the IOFC were AP +3.8 (from bregma), ML +/-2.6, DV -2.9. The location of the all cannula was based on previously published reports (Barrus et al., 2016; Zeeb & Winstanley, 2011). Animals were given a week to recover in their home cage before restarting behavioural testing.

4.2.3 Infusions

Baseline: The infusion procedure was based on previously published research (Winstanley et al., 2003). Once behavioural stability was re-established, animals were given two mock infusions to habituate them to the infusion procedure. Both mock infusions and infusions were performed on a three day cycle. On day one, animals performed a baseline session; on day two, animals were infused with either drug or vehicle, and day three was a rest day during which animals remained in their home cage. The ‘baseline’ condition was a within-subjects design, in which all animals received both drug and vehicle.
During the infusion, animals were gently restrained while their obdurators were removed and 29-gauge injectors were inserted into the guides. These injectors projected 1 mm beyond the end of the cannulae. Once these were inserted, animals were infused with 0.5 µl solution via a dual-channel infusion pump (Harvard Apparatus, Holliston, MA, USA) at a rate of 0.25 µl per minute. Injectors were left in place for one minute following the infusion to allow the solution to diffuse. Injectors were then removed, and autoclaved obdurators were once again affixed to the cannulae. Animals were returned to their home cages for a period of ten minutes to allow the drug to take effect, and then moved to the operant chamber to perform the task. Infusions took place in a procedure room adjacent to the operant behavioural testing room.

Animals in the baseline group received two infusions: vehicle (0.9% saline) and a GABA agonist cocktail composed of the GABA<sub>B</sub> receptor agonist baclofen (Sigma-Aldrich, Oakville, Ontario, Canada) and the GABA<sub>A</sub> receptor agonist muscimol (Sigma-Aldrich, Oakville, Ontario, Canada) (.125 µg of each in .5 µl saline). The order in which the drug was administered was counterbalanced across animals.

**Acquisition**: The infusion procedure for the acquisition group was identical to that of the baseline group, except for the schedule on which the infusions were administered. Animals in this group were given one mock infusion on the last 5CSRTT session, and then given infusions for two consecutive forced-choice sessions and the first four sessions of the full CrGT. This schedule insured that their initial exposure to and thus learning about the contingencies of the task took place only in the context of an infusion. This condition was a between-subjects design, such that animals were split into ‘drug’ and ‘vehicle’ groups; animals in the drug group were always infused with the GABA agonist cocktail, whereas animals in the vehicle group always received saline.
Once all infusions had been administered, animals continued performing the CrGT until they had reached stable baseline behavior.

4.2.4 Drugs
Drugs were purchased from Sigma-Aldrich. Solutions were prepared fresh each day. Baclofen hydrochloride and muscimol hydrobromide were each dissolved separately in 0.9% saline at a concentration of .5mg/ml, then mixed together in equal parts; this produced a final solution with a concentration of .25mg/ml of each drug.

4.2.5 Reward devaluation
Upon completion of baseline inactivations, animals underwent a reward devaluation procedure. Animals were free-fed sucrose pellets in their home cages for a period of 1hr, immediately preceding their normal run time. At the end of this 1 hr period, they were taken from their homecages and put into the operant boxes, where they performed the CrGT as normal.

4.2.6 Histology
Once behavioural testing was completed, animals were anesthetized with inhalable isoflurane and then euthanized via exposure to CO2. The brains were removed and fixed in 4% paraformaldehyde for at least 24hrs, then frozen and sliced on a cryostat in 50 µm slices. These sections were stained with cresyl violet and the location of injector tips were plotted onto standard rat brain sections (Paxinos et al., 1998).

4.3 Results
4.3.1 Histology
Animals were excluded from the experiment if the location of injector tips was determined to be outside of the region of interest. The location of all acceptable placements are depicted in Figure 4-1. None of the animals that received infusions targeting the OFC had to be excluded due to
inaccurate cannulae placement, but three animals died before the experiment was completed. The final numbers included in the analyses were therefore as follows: acquisition- saline: 10, drug: 12; baseline- 15. Of the animals that received infusions targeting the BLA, two animals had to be excluded due to inaccurate cannulae placement, and two died before the experiment was completed. The final numbers included in the analyses were therefore as follows: acquisition- saline: 10, drug: 12; baseline- 13.

4.3.2 Acquisition

OFC: There was no effect of inactivation on choice behaviour when choices were considered individually (Figure 4-2)(Sessions 1-20, Choice x Drug: F_{3,1596} = 1.554, p = .227). However, inactivation of the OFC significantly improved choice score on the rGT during acquisition (Figure 4-3, Table 4-1, Table 4-2)(Sessions 1-20, Drug: F_{1,612} = 7.154, p = .008). Score during acquisition also showed a significant effect of session as well as a drug by session interaction, indicating that all animals learned as training progresses, and that animals with OFC inactivations during the first 4 sessions improved more rapidly than the control group (Session: F_{1,612} = 8.28, p = .045, Drug x Session: F_{1,612} = 8.69, p = .003).

OFC inactivation decreased premature responses (Drug: F_{1,672} = 4.29, p = .039). There was a significant effect of session as well as a drug by session interaction (Session: F_{1,672} = 5.85, p = .016; Drug x Session: F_{1,672} = 6.46, p = .011), indicating that animals made significantly fewer premature responses as they gained familiarity with the task, and that this learning differed between groups.

OFC inactivation increased choice latency and collection latency relative to controls (Choice Latency: Drug: F_{1,624} = 27.00, p < .001; Collection Latency: Drug: F_{1,624} = 5.74, p = .017). For choice latency, there were both session effects and a drug x session interaction (Session: F_{1,624} =
185.61, p < .001; Drug x Session: F<sub>1,624</sub> = 6.68, p = .010). For collection latency, there were only effects of session (Session: F<sub>1,624</sub> = 23.91, p < .001).

For both inactivation and control groups, trials increased as animals learned the task (Session: F<sub>1,637</sub> = 250.24, p < .001), but there were no effects of drug or a drug x session interaction (All F < 2.12, all p > .15). Animals in the inactivation group had a significantly higher rate of omissions than those in the control group, and both groups made fewer omissions on average as training progressed (Drug: F<sub>1,637</sub> = 32.93, p < .001; Session: F<sub>1,637</sub> = 96.34, p = .007; Drug x Session: F<sub>1,637</sub> = 34.34, p < .001).

**BLA:** In contrast to the effects observed in the OFC inactivation cohort, inactivation of the BLA did not significantly affect choice behaviour on the rGT relative to saline controls either when choices were considered separately (Figure 4-2)(Sessions 1-20, Choice x Drug: F<sub>3,1425</sub> = 1.901, p = .267) or when choice score was analyzed (Figure 4-3, Table 4-1)(Drug: F<sub>1,592</sub> = 0.052, p = .819). Furthermore, there were no effects of session on score, indicating that animals’ choice score did not significantly change as training progressed (Session: F<sub>1,592</sub> = 0.029, p = .865).

As with OFC inactivations, BLA inactivations increased premature responses (Drug: F<sub>1,672</sub> = 13.84, p < .001), and both experimental and control animals committed fewer premature responses as training progressed (Session: F<sub>1,672</sub> = 4.71, p = .030; Drug x Session: F<sub>1,672</sub> = 5.21, p = .023).

BLA inactivations did not affect choice latency or collection latency (Choice latency: Drug: F<sub>1,580</sub> = 0.06, p = .814; Collection latency: Drug: F<sub>1,580</sub> = 17.96, p < .001), but animals in both groups were quicker to make a choice as training progressed (Session: F<sub>1,580</sub> = 126.29, p < .001). They were also quicker to collect reward over time (Session: F<sub>1,580</sub> = 17.96, p < .001).
Trials were not significantly affected by inactivation (Drug: $F_{1,484} = 1.58$, $p = .210$), but animals completed more trials as training progressed (Session: $F_{1,484} = 62.57$, $p < .001$). In contrast, BLA inactivation drove an increase in omissions relative to controls (Drug: $F_{1,598} = 6.83$, $p < .001$), and omissions in both groups decreased over time (Session: $F_{1,598} = 69.18$, $p < .001$; Drug x Session: $F_{1,598} = 12.80$, $p < .001$).

4.3.3 Baseline

While the OFC and BLA groups visually appeared to have different choice preferences at baseline, these differences were not statistically significant across all choices (Figure 4-5)(Region x Choice: $F_{3,78} = 1.30$, $p = .281$), nor choice scores (Region: $F_{1,26} = .036$, $p = .851$).

OFC: Inactivation of the OFC at baseline did not significantly affect choice of individual options (Figure 4-5)(Drug x Choice: $F_{3,29} = 0.015$, $p = .631$) or choice score on the CrGT (Figure 4-6, Table 4-2)(Drug: $F_{1,29} = 0.92$, $p = .764$). OFC inactivation did increase choice latency (Drug: $F_{1,29} = 13.11$, $p = .001$), collection latency (Drug: $F_{1,29} = 4.39$, $p = .045$), and omissions (Drug: $F_{1,29} = 19.71$, $p < .001$). Inactivation decreased premature responses (Drug: $F_{1,30} = 7.192$, $p = .012$). Trials were not significantly affected (Drug: $F_{1,29} = 0.578$, $p = .453$).

BLA: Inactivation of the BLA at baseline did not significantly affect choice of individual options (Figure 4-5)(Drug x Choice: $F_{3,33} = 0.014$, $p = .770$) or choice score on the CrGT (Figure 4-6, Table 4-2)(Drug: $F_{1,29} = 0.145$, $p = .707$). However, it affected many other non-choice measures of behaviour on the task. BLA inactivation increased premature responses (Drug: $F_{1,30} = 25.35$, $p < .001$), choice latencies (Drug: $F_{1,29} = 25.61$, $p < .001$), omissions (Drug: $F_{1,30} = 16.04$, $p = .003$), and decreased trials (Drug: $F_{1,29} = 10.24$, $p = .003$) relative to the saline control. Collection latency was not significantly affected by BLA inactivation (Drug: $F_{1,29} = 2.768$, $p = .107$).
4.3.4 Reward devaluation

Reward devaluation did not change animals’ choice preference (Choice: $F_{1,292} = 0.228$, $p = .634$). It did result in increases in choice latency, and decreases in trials (Choice latency: $F_{1,36} = 7.41$, $p = .010$; Trials: $F_{1,36} = 73.98$, $p < .001$). No other variables were significantly affected (All Fs < 1.19, all $p > .282$; Table 4-3).

4.4 Discussion

In the present work, pharmacological inactivation of the OFC during the initial four sessions of the CrGT resulted in a significant improvement in choice that was maintained throughout acquisition. Inactivation of the OFC at baseline did not result in a similar shift in choice preference, indicating that the contributions of the OFC to choice on the CrGT may be most behaviourally relevant when the animal is learning about the contingencies of the task, rather than when it is executing well-learned behaviour. This result differs from the findings of similar work done with the uncued rGT; when the task does not feature salient, win-paired cues, OFC inactivation during acquisition simply delays the development of the optimal choice strategy, but does not otherwise change choice behaviour (Zeeb et al., 2010a). The contrast between these results provides further evidence that activity in the OFC is uniquely recruited by the presence of salient cues (Zeeb et al., 2010b), and suggests that the region is essential to the development of a cue-biased pattern of responding on the CrGT. Furthermore, inactivation of the BLA at either the start of training or during baseline performance did not have a significant effect of choice behaviour on the CrGT, a result that also contrasts with findings from the rGT, where BLA inactivations delayed acquisition and impaired baseline performance (Zeeb & Winstanley, 2011). In sum, these findings suggest that the presence of cues modifies the involvement of both the BLA and the OFC in the development and expression of this form of complex cost-benefit decision making, and that activity of OFC may promote the development of
disadvantageous, cue-biased decision making. These findings clarify the neuroanatomical underpinnings of decision making on the CrGT and in the context of salient cues more generally, and suggest that the OFC should be the target of future work to clarify the regional pharmacology and connectivity that drives these effects.

Unfortunately, the BLA-targeted cannulations of the acquisition group could not be histologically verified because the sections taken were too anterior, and the remaining tissue was discarded before the error was detected. It is therefore possible, however unlikely, that there were errors during surgery and the BLA-targeted infusions did not, in fact, target the BLA. Given that we had an error rate of 13% in the BLA-targeted baseline group which we were able to verify, we expect that few, if any, animals from the acquisition group would have had to be excluded.

Unlike the baseline inactivations performed in this study, or most other pharmacological work performed with the CrGT (Adams et al., 2017; Barrus & Winstanley, 2016), the acquisition inactivations followed a between-subjects design in which animals were split into either an inactivation group or a saline group. This design can be weaker than the within-subjects design we usually employ, in which animals serve as their own controls. It is therefore possible, however unlikely, that the animals in the OFC acquisition inactivation group were categorically different from those in the control group and the BLA acquisition inactivation group, and would have exhibited more optimal choice on the CrGT even in the absence of pharmacological inactivation. We see this possibility as unlikely. Several hundred Charles River sourced Long-Evans rats have been trained by different researchers on the CrGT since its development in cohorts that vary in size from 8-80 animals; the mean score in these cohorts reliably falls around zero and is normally distributed. The probability that the OFC acquisition inactivation group
represent some manipulation-independent deviation from this pattern seems incredibly unlikely. Furthermore, it is not possible to perform an acquisition manipulation using a within-subjects design. By definition, subjects can only learn the specific contingencies on the CrGT once. To the best of our knowledge, there is no way they could unlearn and then relearn the task to serve as their own controls.

We must also consider the possibility that the sustained effects of chronic infusions in the OFC acquisition infusion group could be due to long-lasting structural or functional damage caused by repeated infusions in the area, rather than the inactivation of the region during the first four sessions. We feel this concern is warranted but has been properly controlled for. First, we limited the number of infusions each animal received in order to minimize tissue degradation. Animals were subject to no more than 3 µl of infused fluid, while other researchers have successfully infused up to 50 µl (Liebsch et al., 1995). Second, an equal number of control animals received an identical number and volume of saline infusions into the OFC, and these animals exhibited the same pattern of choice as those who received saline infusions to the BLA. Furthermore, when checking cannula placement, there were no differences in tissue displacement between saline- and GABA$_{AB}$ agonist-treated animals. While we believe that this concern has been adequately controlled for in the present work, future work could make use of minimally invasive techniques such as DREADDs (Krashes et al., 2012) or optogenetics (Zhang et al., 2005) that do not require repeated infusions into the region of interest in order to downregulate activity.

The robust improvement in choice driven by inactivation of the OFC during CrGT acquisition is particularly interesting when it is compared to the aforementioned lack of effect of OFC on choice on the uncued rGT (Zeeb & Winstanley, 2011).
effects suggests that the OFC is both uniquely recruited by the presence of salient cues, and also plays a role in biasing choice towards the cue-paired, risky options on the CrGT. These findings are not without precedent. Work done with a delay discounting paradigm has shown that the effects of OFC inactivation were partially dependent on the presence or absence of a cue illuminated throughout the delay to delivery of the larger-later reward, and that dopamine antagonism within the OFC reduced the impact of this cue on choice behaviour (Zeeb et al., 2010b). On the rodent betting task (rBT), a subset of animals show a mathematically irrational aversion to uncertainty as the available reward increases. These animals are termed “wager-sensitive”, while the remainder are “wager-insensitive” (Cocker et al., 2012). The OFC appears to play a critical role in guiding this irrationally risk-averse choice preference; inactivation of the OFC in wager sensitive animals leads to an increase in their choice of the uncertain options at the larger bet sizes, effectively ameliorating the bias (Barrus et al., 2016). In contrast, wager-insensitive animals do not show a change in choice behaviour as a result of OFC inactivation, suggesting that the OFC is critical specifically when animals employ a choice strategy that relies on subjective value judgement about each option, rather than a judgement based on the objective mathematical return.

Considerable evidence supports a role for the OFC in monitoring and updating the subjective value of rewards and outcomes (see Kringelbach & Radcliffe, 2005 for review); for example, OFC neurons modulate their firing rates in response to the devaluation of a preferred reward (Kringelbach et al., 2003). Collectively, these findings indicate that one factor contributing to the general impairment in choice observed on the CrGT relative to the uncued rGT may be the cue-driven recruitment of the OFC; once involved in the development of a decision making strategy, it promotes choice of the tempting, saliently-cued options. A
preference for these risky options may represent a reliance on a subjective choice bias over more objective assessments of the value of options, much like that exhibited in wager-sensitive animals on the rBT. Another way of framing this may be that the inactivation of the OFC inoculated them against the motivational value of the cues, and they instead developed a decision making strategy based on some other goal, such as reward maximization. Some research has shown that healthy human subjects with intact OFCs adopt a more disadvantageous pattern of decision making than subjects with acute OFC damage, perhaps because the OFC promotes ‘myopic’ choice that is in part based on the emotional responses to the task rather than a more objective assessment of expected returns (Weller, Levin, Shiv, & Bechara, 2007). In this example, subjects with intact OFCs were more likely to choose a certain option with lower expected utility than an uncertain option with higher expected utility. In contrast, OFC-lesioned subjects adopted a more objective choice strategy of choosing the uncertain but advantageous option more frequently. This is not unlike the effects described here, where normal controls appear to be sensitive to the ability of salient cues to disrupt optimal decision making, as evidenced by their suboptimal choice score. OFC inactivation may have thus lead to more advantageous choice on the CrGT by lessening the impact of these cues relative to more objective assessments of how much sucrose choice of each option would return. Future work targeting specific neuronal subpopulations within the OFC could provide more information about the pharmacological correlates and inter-regional connectivity that is responsible for these effects.

Reward devaluation procedures evaluate the extent to which instrumental behaviour is goal-directed by comparing normal task performance against performance once the reward associated with that task has been devalued (Balleine & Dickinson, 1998). In theory, a drop-off
in responding for a reward following that reward’s devaluation indicates that the animal is no longer motivated to achieve that reward, indicating that the behaviour is goal directed. If behaviour was not modified by reward devaluation, it can be taken as an indication that behaviour has become habitual or at least inflexible, rather than goal-directed (see Dickinson et al., 1994 for review). Conducting a reward devaluation procedure in the present experiment was of interest to us in part because of the lack of effect of baseline inactivations on choice behaviour, particularly in the OFC group. Given the robustness of the effect of acquisition inactivation in this group, it was surprising that repeating the same manipulation at baseline did not result in statistically significant changes in choice behavior. Furthermore, the lack of effect of BLA inactivation during either acquisition or performance was unexpected in the context of the robust effects observed on the rGT by Zeeb et al. Observing these null effects prompted us to employ this reward devaluation manipulation, in order to clarify the apparent inflexibility of well-learned CrGT behaviour. It seemed possible that the null effects of baseline inactivation reflected a general resistance of baseline CrGT choice to manipulation. Previous reward devaluation work with the uncued rGT found that reward devaluation changed animals’ choice preferences, increasing choice of P1 and decreasing P2, as well as slightly increasing choice of P3 and P4 (Zeeb & Winstanley, 2013). In contrast to these effects, the same manipulation did not affect choice on the CrGT, suggesting that well-learned behaviour on this task is less flexible in comparison. As salient cues represent the only structural difference between the tasks, these results suggest that cues may accelerate the development of an inflexible pattern of responding on the CrGT. While resistant to manipulation, learned behaviour on the CrGT cannot be said to be habitual because devaluation affected some non-choice measures, including latencies and trials, whereas truly habitual responding should be entirely immune to devaluation. Nonetheless,
the inflexibility of CrGT behaviour superficially resembles substance use disorders (SUD) and other addiction-like disorders where behaviour is resistant to change and persists despite adverse consequences.

Researchers have argued that the transition to inflexible or compulsive responding which is a central feature of addictive behaviours represents a transition from prefrontally controlled decision-making processes to striatally controlled processes, and that drug associated cues promote and maintain behaviour during this transition (Everitt & Robbins, 2005). In human subjects, the ability of drug paired cues to promote drug craving is well documented (Volkow et al., 2006; Childress et al., 1993; Grimm et al., 2001). Attention to gambling related stimuli predicts gambling frequency and susceptibility to addiction (Grant et al., 2014), suggesting that cues may alter the decision-making processes that underlie the transition from choice to more inflexible responding. Further work with the CrGT could examine the contribution of areas such as the dorsal striatum that are thought to be recruited by these patterns of inflexible behaviour in order to determine what role, if any, they play in well learned behaviour on the CrGT. There is an argument to be made that behaviour on the CrGT is in some ways more flexible than behaviour on the rGT, in that choice at baseline is distributed more evenly across each of the four options. This is a point worth consideration. While animals performing the rGT appear to largely subscribe to a strategy of reward maximization, the broad distribution of choice scores on the CrGT suggests that animals are applying disparate strategies that are not principally geared towards aggregating as many sucrose pellets as possible. However, it is a slightly different line of investigation from that described in previous paragraphs, which pertain to the specific inability of pharmacological and surgical manipulations to modify well-established choice
behaviour on the CrGT, rather than the broader question of how animals come to establish said choice preference on the task.

The finding that BLA inactivations did not significantly affect choice on the CrGT also contrasts with work on the rGT in which lesions of the BLA delayed task acquisition and impaired baseline performance (Zeeb & Winstanley, 2011). The relative inflexibility of baseline choice may help explain the null effects of inactivations at that stage, but do not explain why acquisition behaviour was not affected. Mechanistically, Zeeb et al (2011) theorized that the BLA signalled the adverseness of the punishment associated with the riskier options, and that BLA damage rendered animals insensitive to these punishment signals. This theory fits in with a larger universe of fear conditioning literature that posits a role for the BLA in detecting and promoting avoidance of aversive stimuli (Phillips et al., 1992; LeDoux, 2000 for review). The closest human parallel to Zeeb et al’s findings may be work done with the Iowa Gambling Task (IGT), the human precursor to the rGT; amygdala-lesioned patients likewise had elevated choice of risky options relative to healthy controls, and failed to generate skin conductance responses both before making risky choices and upon experiencing the outcomes of their choice (Bechara et al., 1999). Furthermore, inhibition of the BLA leads animals to shift their preference from smaller but safer rewards to larger rewards that are paired with some likelihood of a footshock punishment (Orsini et al., 2017; Piantadosi et al., 2017), indicating again that the BLA guides choice behaviour in the context of risk.

It is puzzling then that these inactivations had no effect on behaviour on the present task, though there are several possible explanations for why this did not occur. For example, these findings may suggest that loss avoidance does not guide behaviour early in acquisition, and thus the BLA is less relevant at this stage. Early in acquisition, animals select P1 as frequently or...
nearly as frequently as they do P2 on both the rGT and CrGT (Barrus & Winstanley, 2016; Zeeb et al., 2009). While this option presents the lowest risk and duration of punishments, it is also the most frequently rewarded option. Seeking frequently rewarded options may play a greater role in determining choice behaviour on the CrGT than avoiding timeout punishments early in acquisition, making input from the BLA less relevant in determining choice behaviour. However, given that the presence of reward-concurrent cues biases choice towards P3 and P4, it would not have been surprising if the more frequent experience of punishing time-outs as a result of such a maladaptive choice strategy more aggressively recruited the BLA into the decision-making process.

As discussed previously, the BLA also responds to reward-predictive cues (Ambroggi et al., 2008) and distinct populations of neurons within the BLA appear to encode either fear or reward (Redondo et al., 2014). A “dual valence model” of BLA activity proposes that these distinct populations of neurons may demonstrate a mutually inhibitory relationship. These parallel and competing circuits may allow the BLA to facilitate both avoidant and approach behaviour depending on inter-regional inhibition of one circuit or the other (Janak & Tye, 2015). It seems plausible that the addition of salient cues promotes greater responding in these reward-encoding neurons, and this mutual inhibition attenuates the BLA’s signalling of either appetitive or aversive stimuli relative to the uncued paradigm, making it less behaviourally relevant to the CrGT.

While inactivation with GABA agonists is a useful technique for establishing whether or not a given region is involved in a given behaviour, it is a relatively gross manipulation which does not provide nuanced information about the nature of that region’s involvement. Even relatively simple techniques such as the infusion of receptor-specific ligands into these regions
could clarify the pharmacology of these effects. It is possible that the effects reported here are not dependent on general OFC activity but instead the activity of specific subpopulations of neurons within the OFC that share common pharmacological characteristics or projection patterns. (Kahnt et al., 2012). Furthermore, pharmacological inactivation provides no information about the temporal nuance of the region’s involvement on the scale of an individual decision, or even the progression of decisions across the course of a session. The shift towards optimal choice described above may be due to activity of the OFC during the receipt of reward, or during the experience of punishment. More recent techniques such as DREADDs and optogenetics allow researchers to target these neuronal subpopulations, projection patterns and temporal windows with increased precision. Future work with the CrGT could employ them in order to determine what neuronal characteristics of the OFC promote the development of cue-biased behaviour, and if there are particular features of the task that the OFC sensitive to.
<table>
<thead>
<tr>
<th></th>
<th>OFC</th>
<th>BLA</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>47.00 +/- 2.52**</td>
<td>10.60 +/- 4.11</td>
<td>17.94 +/- 2.68</td>
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<tr>
<td>Premature Responses</td>
<td>19.82 +/- 0.78*</td>
<td>21.84 +/- 1.17***</td>
<td>24.05 +/- 0.74</td>
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<td>Choice latency</td>
<td>2.56 +/- 0.06***</td>
<td>2.24 +/- 0.07</td>
<td>2.21 +/- 0.05</td>
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<tr>
<td>Collection latency</td>
<td>1.18 +/- 0.04**</td>
<td>1.14 +/- 0.07</td>
<td>1.02 +/- 0.05</td>
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<tr>
<td>Trials</td>
<td>81.13 +/- 2.01</td>
<td>86.74 +/- 2.48</td>
<td>82.98 +/- 1.64</td>
</tr>
<tr>
<td>Omissions</td>
<td>4.67 +/- 0.45***</td>
<td>3.47 +/- 0.40***</td>
<td>2.37 +/- 0.18</td>
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.05 = *, .01 = **, .001= ***

**Table 4-1:** Behavioral measures during acquisition. Data shown are mean ± SEM.
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<th>OFC</th>
<th>BLA</th>
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<tr>
<td>Score</td>
<td>2.23 +/- 15.86</td>
<td>-4.47 +/- 15.42</td>
</tr>
<tr>
<td>Premature Responses</td>
<td>12.77 +/- 3.29*</td>
<td>24.14 +/- 3.83</td>
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<tr>
<td>Choice latency</td>
<td>2.92 +/- 0.24***</td>
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<td>Collection latency</td>
<td>1.06 +/- 0.13*</td>
<td>0.77 +/- 0.06</td>
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<td>Trials</td>
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<tr>
<td>Omissions</td>
<td>9.4 +/- 1.84***</td>
<td>1.25 +/- 0.45</td>
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.05 = *, .01 = **, .001= ***

Table 4-2: Effects of regional inactivation on behaviour at baseline. Data shown are mean ± SEM.
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<thead>
<tr>
<th></th>
<th>Naive</th>
<th>During devaluation</th>
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<tr>
<td>Score</td>
<td>15.95 +/- 3.98</td>
<td>18.22 +/- 3.86</td>
</tr>
<tr>
<td>Premature Responses</td>
<td>16.29 +/- 1.54</td>
<td>17.83 +/- 1.45</td>
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<td>Choice latency</td>
<td>1.87 +/- 0.10</td>
<td>2.14 +/- 0.11**</td>
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<td>Collection latency</td>
<td>.88 +/- 0.04</td>
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<td>Trials</td>
<td>76.46 +/- 3.29</td>
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<td>Omissions</td>
<td>1.27 +/- 0.28</td>
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.05 = *, .01 = **, .001 = ***

Table 4.3: Effects of reward devaluation manipulation on behaviour. Data shown are mean ± SEM.
Figure 4-1: Location of acceptable placements of cannula within the OFC. A) Location of cannula tips in the acquisition group. B) Location of cannula tips in the baseline group.
Figure 4-2: Choice of each option over sessions 1-20. Inactivation of either the BLA or OFC did not significantly affect choice of individual options relative to saline controls (BLA: Choice x Drug: $F_{3,1596} = 1.342$, $p = .267$; OFC: Choice x Drug: $F_{3,1596} = 1.475$, $p = .227$). While animals in the OFC-inactivated group do appear to choose P3 less frequently than the BLA and saline groups (panel C), this difference is not significant, perhaps because of high between subject-variability (OFC: P3: Drug: $F_{1,28} = 1.855$, $p = .184$). Data shown are mean ± SEM.
Figure 4-3: Score over sessions 1-20. As training progressed, animals in the OFC inactivation group demonstrated a shift in preference away from the disadvantageous options, resulting in an increase in score over time. They maintained this strong preference for these options for the duration of acquisition. In contrast, animals in the saline group and those in the BLA inactivation group maintained a much more slight preference for the advantageous options, and the average score in these groups was closer to zero than that of the OFC group. Data shown are mean ± SEM. * denotes p < 0.05.
Figure 4-4: Individual scores at session 20. Animals in the OFC inactivation group were more likely to demonstrate a preference for the more advantageous options than animals in either the saline group or the BLA inactivation group. Furthermore, the distribution of scores differed between groups. Scores in the OFC group were skewed towards the upper limit of the scale, whereas scores in the BLA group and the saline group were more evenly distributed across the scale’s entire range. Data shown are mean score.
Figure 4-5: Effect of baseline inactivation on choice option by region. Inactivation of the OFC (panel A) or the BLA (panel B) did not significantly affect choice of any option on the task (OFC: Choice x Drug: $F_{3,39} = 0.631, p = .599$; BLA: Choice x Drug: $F_{3,33} = 0.378, p = .770$). Data shown are mean ± SEM.
Figure 4-6: Effect of inactivations on score by region. While the OFC and BLA groups visually appeared to have different choice preferences at baseline, these differences were not statistically significant across choice scores (Region: $F_{1,26} = .036$, $p = .851$). Data shown are mean ± SEM.
Fig 4-7: Effect of reward devaluation on choice of individual options as well as score. Reward devaluation did not significantly affect either choice of individual options (Panel A, Devaluation: $F_{1,36} = 2.225, p = .145$; Choice x Devaluation: $F_{3,108} = 2.046, p = .112$) or score (Panel B, Devaluation: (Drug: $F_{1,29} = 0.145, p = .707$)). This lack of effect suggests that learned behaviour on the CrGT is inflexible or otherwise resistant to manipulation. Data shown are mean ± SEM.
Chapter 5: Chronic DREADDs inhibition of the nucleus accumbens core, but not shell, transiently improves learning on a rat Gambling Task

5.1 Introduction
Gambling disorder (GD) is a DSM-V recognized behavioural addiction defined by a loss of control over gambling behavior (Potenza, 2008; Reilly & Smith, 2013). Research into the neurobiological mechanisms that promote GD in humans is limited by practical and ethical constraints, but animal models enable researchers to perform experiments which isolate the contributions of specific brain regions and neurotransmitters in ways that are not possible in human research (Cocker & Winstanley, 2015; Orsini et al., 2015; Winstanley, 2011b). Our laboratory has developed an animal model of gambling behaviour called the rat Gambling Task (rGT), in which animals are able to choose between several options associated with different schedules of reward and punishment (Zeeb et al., 2009). The optimal strategy is to favor the options associated with small, frequent reward and small, infrequent punishment. Work with this paradigm has informed our understanding of the contributions of cortical and subcortical regions to performance on the task, as well as the neurochemistry governing gambling-like behavior (Barrus, Hosking, Zeeb, Tremblay, & Winstanley, 2015; Zeeb et al., 2015; Zeeb et al., 2009; Zeeb & Winstanley, 2013). However, work to date has not yet examined the role of the striatum in this form of behaviour, despite the region’s well documented involvement in various forms of behavioural output, including reward-related decision making (see Floresco, 2015 for review).

The striatum can be subdivided into dorsal and ventral regions, based on functional and structural heterogeneity (Jongen-Rêlo, Voorn, & Groenewegen, 1994). The ventral striatum, or nucleus accumbens (NAc) has extensive inputs from limbic, midbrain, prefrontal and temporal regions and projects to subcortical motor regions, making it perfectly positioned to take input from a variety of regions which are necessary for goal-directed behaviour and translate it into
motivationally relevant motor output (Brog et al., 1993; Heimer et al., 1991; Sesack & Grace, 2009). For this reason, it is commonly referred to as the limbic-motor interface. The NAc is comprised of two primary subregions—the core (NAcC) and shell (NAcS), and these appear to make distinct contributions to behaviour.

The NAcC appears to guide motivated behavior towards desired options (Floresco, 2015). Excitation in the NAcC is correlated with latency to approach a reward, suggesting that it plays a causal role in invigorating approach and engagement with reward and reward-related stimuli (du Hoffmann & Nicola, 2014). Additionally, NAcC inactivations increased the latency to make a response to a conditioned stimulus, reduced the rate of lever pressing for reward, and decreased checking for reward, collectively indicating that the NAcC is necessary to facilitate task engagement (Ambroggi et al., 2011).

In contrast, the NAcS appears to be important for inhibiting inappropriate behaviours that would otherwise hinder appropriate goal-directed action (Floresco, 2015). In one example, inactivation of the NAcS increased responding in a test of cue-induced reinstatement, indicating that the region normally plays a role in suppressing behavioral responding to irrelevant or non-rewarded stimuli (Di Ciano et al., 2008). Furthermore, inactivation of the NAcS increased responding to a cue that was explicitly not paired with reward, again suggesting that the shell plays a critical role in response inhibition to less relevant stimuli (Ambroggi et al., 2011). The NAcS receives extensive projections from cortical regions including the PrL, IL and OFC (Brog et al., 1993), and the ability of the NAcS to suppress behaviour that is irrelevant or otherwise inappropriate could conceivably be related to the higher-order input from these afferents.
Given these well-documented contributions of the striatum to motivated behaviour, its role to rGT performance are of particular interest to us. We therefore propose to chemogenetically inhibit both the NAcS and NAcC, both during acquisition of the task and once baseline performance has been established. We hypothesized that inhibiting the NAcS would lead to riskier, more disadvantageous behaviour on the CrGT, while inhibition of the NAcC would lead to an overall decrease in task engagement.

5.2 Methods
5.2.1 Subjects

Subjects were 48 male Long-Evans rats (Charles Rivers Laboratories, St. Constant, Quebec, Canada) weighing 250-275g at the time of arrival at the animal facility.

5.2.2 Surgery

Subjects underwent surgery prior to behavioural testing. 1 ul of AAV was infused at a rate of 0.2 ul/minute using 32 G stainless steel injectors (Plastics One, Roanoke, VA), PE tubing (Instech), and 10 ul syringes (Hamilton). Injectors were left in place for 10 minutes to ensure solution fully diffused from the injector tip. Inhibitory DREADD pAAV5-hSyn-HA-hM4D(Gi)-mCherry7 (UNC Vector Core, 112 Durham, USA; titer ≥ 3×10¹² vg/mL) was bilaterally injected into either the NAcC (n = 24) or NAcS (n = 24). The injection site for the NAcC was AP: +1.2, ML: ±1.8, DV: −7.1, while the injection site for NAcS was AP: +1.6, ML: ±1.1, DV: −7.9. These coordinates were based on previously published reports (Piantadosi, Yeates, Wilkins, & Floresco, 2017) and modified following a round of pilot surgeries. Animals recovered for a minimum of six weeks before beginning behavioural training in order to allow sufficient time for viral expression.
5.2.3 Behavioural testing

Animals were trained in the manner described in Chapter 2: general methods, up to the end of the 5-CSRTT training.

5.2.4 Drugs

Clozapine-n-oxide (CNO; Toronto Research Chemicals, Toronto, Canada) was prepared fresh daily. Doses were calculated as the salt and dissolved in vehicle prior to injections. CNO was dissolved at a volume of 1mg/ml in 6% DMSO and 0.9% sterile saline.

5.2.5 Acquisition

The first round of CNO administration began 6 weeks after viral infusions. We employed a between-subjects design, where animals were split into ‘drug’ (n = 24; NAcC = 12, NAcS = 12) and ‘vehicle’ (n = 24; NAcC = 12, NAcS = 12) groups; animals in the drug group always received CNO during an acquisition injection, and animals in the vehicle group always received vehicle during an acquisition injection.

Animals began receiving injections at the start of forced-choice rGT training. For this procedure, animals were removed from their home cage and gently restrained and injected with 1mg/kg solution via the intraperitoneal (i.p.) route. They were then returned to their home cage for 30 minutes to allow the drug to take effect before being transferred to the operant boxes for the start of behavioural testing. These injections took place for all five consecutive forced-choice sessions and the first five sessions of the full rGT, resulting in ten injections total. This injection schedule insured that their initial exposure to and thus learning about the contingencies of the rGT took place only in the context of an injection.

Once these injections were complete, animals continued performing the rGT until they reached stable baseline behavior (session 25).
5.2.6 Baseline:
Baseline injections began once animals had achieved stable baseline responding, defined as a nonsignificant effect of session and choice x session interaction on a repeated-measures ANOVA across the previous three sessions. The order in which drug doses were administered was determined by a Latin-square design. Each drug was administered in 3 d 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. To prevent any potential carryover effects, animals were given a washout period between drugs of at least 1 week. During this period, they were tested on the task.

Animals received intraperitoneal (i.p.) injections of vehicle (6% DMSO in saline), 0.3 mg/kg, 1.0 mg/kg and 3.0 mg/kg CNO. As before, these injections took place 30-min before the start of the behavioural testing.

5.2.7 Histology
At experimental endpoint, rats were sacrificed by transcardial perfusion. Rats were injected with 120 mg/kg ketamine and 15 mg/kg xylazine i.p., and then perfused with cold 10% phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were extracted and stored in 4% PFA for at least 24hrs before being transferred to 30% sucrose solution. The brains were then frozen and sliced at 50 μm on a cryostat. Sections of the striatum were taken and stored as free-floating slices in PBS. Sections were processed with 1μg/mL 4’,6-diamidino-2-phenylindole (DAPI) in PBS and incubated for 15 minutes immediately before being mounted onto gelatin-coated glass slides, and cover-slipped with VectaShield anti-fade Mounting Medium (Vector Laboratories, Burlingame, CA, USA). Expression of mCherry was confirmed using an AxioZoom V16 microscope (Zeiss, Germany).
5.3 Results

5.3.1 Histology

Animals were excluded from the experiment if the location of DREADDs expression was determined to be outside of the region of interest. Representative examples of DREADDs expression are shown in figure 5-1. Two animals from the NAcS group and one from the NAcC group were excluded because expression was poor or incomplete. Final group sizes were as follows: NAcS = 22; NAcC = 23.

Acquisition

Choice effects

As expected, there was a significant effect of session on choice (Session: F_{1,805} = 28.74, p < .001), indicating that animals developed choice preferences as training progressed. CNO administration during acquisition also differentially affected choice behaviour on the rGT in a region-dependent manner (region: F_{4,192} = 2.61, p = .037; drug: F_{4,192} = 4.64, p = .001; region x drug: F_{4,192} = 3.09, p = .017; region x drug x choice = F_{2,3242} = 3.965, p = .019).

In keeping with previous reports (Zeeb & Winstanley, 2011; Zeeb & Winstanley, 2013), animals receiving vehicle injections progressively increased choice of P2, and decreased choice of P1, while preference for P3 and P4 started lower and decreased slightly as training progressed (Choice of P1: Session: F_{1,402} = 35.04, p < .001; Choice of P2: Session: F_{1,402} = 81.34, p < .001; Choice of P3: Session: F_{1,402} = 17.55, p < .001; Choice of P4: Session: F_{1,402} = 21.93, p < .001). During inhibition of the NAcC, development of this optimal strategy was accelerated, such that animals selected P1 less often in favour of P2 (Figure 5-2, table 5-1) (Drug x choice F_{1,1592} = 6.084, p = .014; Sessions 1-5: Choice of P1, Drug: F_{1,96} = 6.68, p = .011; Choice of P2, Drug: F_{1,96} = 12.79, p < .001), and these effects on choice were not observed in the CNO-treated NAcS.
group (Region x choice: F_{1,1624} = 4.679, \ p = .031). Choice of P1 also remained below that of vehicle-treated rats for the first five drug-free sessions, after which choice in the control group changed to match that of CNO-treated rats (Sessions 6-10: Choice of P1, Drug: F_{1,91} = 6.05, \ p = .016). Furthermore, the CNO group briefly showed elevated choice of P4 (Figure 5-2)(Sessions 11-15: Choice of P4: Group F_{1,98} = 5.74, \ p = .019). Choice of P3 did not differ as a result of CNO administration (figure 5-2; All F < 2.32, all \ p > .131).

In contrast to these effects, CNO did not affect choice in the shell group (Table 5-2, All F < 1.94, all \ p > .10).

**Non-choice effects**

CNO did not have significant effects on non-choice variables during drug administration, but did appear to decrease latencies later in acquisition (Table 5-3). Both core and shell groups had shorter choice and collection latencies during the last ten sessions of acquisition (Choice latency: Sessions 11-15: Group F_{1,39} = 4.38, \ p = .037; Sessions: 16-20: group: F_{1,39} = 5.51, \ p = .020; Collection latency: Sessions 11-15: Group: F_{1,39} = 12.84, \ p = .011; Sessions: 16-20: Group: F_{1,39} = 11.81, \ p = .002). There was an effect of group on trials completed across the last five session of acquisition, which was driven by a transitory increase in animals treated with NAcS administered CNO (Trials: S16-20 session F_{1,244} = 3.90, \ p = .049). Premature responses and omissions were not significantly affected by inhibition of either region (All F < 3.37, all \ p > .068).

**Baseline**

**Choice effects**
CNO administration did not affect choice once the task had been learned (Table 5-4; Figure 5-3)(All F < 1.94, all p > .11).

**Non-choice effects**

CNO did lead to a dose-dependent increase in collection latency across both shell and core groups in animals with DREADD expression (Dose: $F_{1,167} = 4.33, p = .04$). No other non-choice variables were significantly affected (All F < 2.94, all p > .09).

**5.4 Discussion**

Chemogenetic inhibition of the NAc core during acquisition of the rGT appeared to accelerate development of the optimal strategy, caused animals to shift their choice towards P2, the option that returns the most reward, earlier in training, and away from P1, an option which delivers smaller, less frequent punishments but also smaller rewards. In contrast, inhibition of the core did not affect choice once the task had been learned, though this manipulation did result in greater latencies to make a choice and collect reward. Inhibition of the NAc shell did not affect choice behaviour on the rGT, regardless of whether this occurred during acquisition or at baseline. These results suggest that, although well-learned behaviour on the rGT is not critically dependent on either the NAcC or the NAcSh, the NAcC is at least partially responsible for the bias towards P1 evident early on in the task, which must be overcome if animals are to truly maximise the reward earned.

CNO-modulated DREADDs technology has come under scrutiny due to work by Gomez et al. While CNO is inactive and inert, it can be readily metabolized by the liver into clozapine, a second generation antipsychotic from which CNO is derived. Clozapine readily crosses the blood-brain barrier, and shows high affinity for DREADDs hM3Di receptors (Gomez et al.,
Furthermore, low doses of clozapine are able to exert DREADDs-mediated behavioural effects (Gomez et al., 2017). This is potentially concerning, as clozapine binds to select dopaminergic, serotonergic and noradrenergic receptors as well as DREADDs (Solanki et al., 2007), and the possibility of nonspecific CNO effects could potentially complicate the interpretation of highly-targeted DREADDs experiments. However, the reported effects were obtained using a less conservative methodology than the one we employed in the present study. A dose of 10mg/kg CNO was necessary to evoke putatively clozapine-mediated locomotor effects (Gomez et al., 2017), while the acquisition effects we observed in the present study were obtained with a dose of 1.0mg/kg. Furthermore, these locomotor effects were observed between 2-3 hours after CNO injection, while behavioural testing in the present work was complete only an hour after CNO injection. Nonetheless, these recent findings suggest that future work should consider the limitations of CNO-mediated DREADDs and employ designs that control for these off-target effects.

One potentially serious caveat of this work is the lack of empty vector control. Work employing DREADDs methodology typically include a control group which has received treatment with a so-called empty vector to ensure that any observed effects of CNO administration are due to the intended action of CNO on synthetic receptors, rather than unintended, off-target effects. While this experiment failed to include such a control, we believe this is mitigated by the null effects of CNO administration on choice behaviour in the NAcS group. While this is not equivalent to the inclusion of empty-vector controls, the contrast in choice effects between the NAcC and NAcS groups does indicate that the observed effects are due to the action of CNO on DREADDs receptors in the NAcC, not a general effect of CNO administration. Were CNO administration sufficient to evoke the observed pattern of choice, we
would expect to see this effect across both groups that had been given CNO. As choice
behaviour during acquisition was modified by CNO only in those animals with DREADDs
expression in the NAcC, we can be reasonably confident that our effects are NAcC specific.
Nonetheless, we regret the error in methodology and our future work employing DREADDs will
include empty vector controls to mitigate these concerns.

Previous work in our lab has demonstrated that inactivation of the NAcC with GABA_{A/B}
agonists results in task disengagement on a cognitive effort task (Silveira, Tremblay, &
Winstanley, 2018). We have observed similar effects on the rGT. In this unpublished work,
GABA_{A/B} inactivation of the NAcC during acquisition and again at baseline leads to a near-total
disengagement with the rGT, such that animals completed very few, if any trials. In the present
work, CNO administration did not reduce trials completed in the NAcC group, either during
acquisition or at baseline, let alone disengagement with the task. Furthermore, GABA_{A/B}
inactivation of the NAcC did not lead to lasting, post-manipulation differences in choice
behaviour relative to a control group, which contrasts with the significant shift in choice
observed in the present work. These differences between the behavioural effects of GABA
agonists and the chemogenetic manipulations reported here suggests that the effect of CNO
administration on NAcC function may be more subtle than the complete inhibition that GABA
agonism produces; while infusion of GABA agonists should result in inhibition of most cells in
the targeted region, CNO administration would inhibit only those cells expressing the hm4D(i)
receptors. The rate of expression varies between individual animals and across experiments, and
this variation can change the strength of the effects evoked by CNO (Roth, 2016). The shift in
choice behaviour towards the best option shown here may therefore reflect some attenuation in
core activity, rather than wholesale inactivation of the region which precludes task performance.
While a preference for P2 is the optimal pattern of choice on the rGT, elevated choice of P1 is reliably observed during acquisition, and can also be evoked via administration of amphetamine (Barrus & Winstanley, 2016; Zeeb et al., 2009). Zeeb et al theorized that this amphetamine-driven shift towards P1 is due to a dopamine-dependent amplification of the salience of the longer and more frequent losses associated with P2 (Zeeb et al., 2009). Work with a sign-tracking paradigm has found that dopamine antagonism in the NAc selectively reduces approach to reward-predictive cues, and this has been taken as evidence that NAc dopamine may play a pivotal role in the attribution of incentive salience to important environmental stimuli (Saunders et al., 2012; Floresco, 2017). Hyperpolarization of NAcC neurons via inhibitory DREADDs receptor activation could have rendered the NAcC less sensitive to dopaminergic regulation and thus disrupted the incentive salience attribution to P2-associated losses, driving a shift towards this choice option. If this mechanism explains the tradeoff between choice of P1 and P2, it suggests that loss aversion may guide choice early in acquisition of the rGT before other considerations (such as reward maximization) come to dominate behaviour. Indeed, in order for animals to choose optimally, they must learn to tolerate the increased frequency and duration of the losses associated with P2, foregoing the more frequent but smaller rewards available on P1. Lesions of the NAcC have been shown to increase impulsive choice on a delay discounting paradigm, and researchers argued that the NAcC maintains a representation of the larger reward, enabling subjects to overcome the cost associated with that choice (Cardinal et al., 2001). This effect appears to contrast with the findings described here where NAcC-inactivated animals become less ‘impulsive’ in that they choose the less frequently rewarded but ultimately more advantageous option more than normal controls. While the rGT and delay discounting tasks are similar in that they can both be taken to provide
some measure of impulsivity, they are designed to track different subsets of behaviour within this broader constellation of “impulsive choice”. It could be argued that the proposed role of the NAcC in the delay discounting task as described by Cardinal et al. is not too different from that we describe here; in both tasks, the NAcC contributes to attribution of salience, be that the larger reward in the delay discounting task and the punishment in the rGT.

The lack of effects of NAcC inactivation on baseline behaviour suggests that the region does not make significant contribution to choice on the rGT in well-trained animals. As suggested above, the essential contribution of the NAcC in this context may be to highlight the loss associated with each option, and a well-learned pattern of behaviour may be dominated by other considerations. In the aforementioned sign-tracking paradigm, inactivation of the NAcC had no effect on the well-learned approach behaviour of goal-tracking animals (Saunders et al., 2012). Well-learned behaviour on the rGT is still goal-directed, as demonstrated by the effects of reinforcer devaluation (Zeeb et al., 2013), therefore the null effects of NAcC inactivation cannot be attributed to the fact that behaviour has become purely habitual. While the salience of punishments may guide behaviour in acquisition, the preference for P2 implies that baseline behaviour is driven by reward maximization, and this reward maximization does not appear to depend on input from the NAcC. Inactivation of the NAcC did increase the latency to make a choice and collect their reward, a finding which corresponds to the theory that the NAcC is essential to instigating approach behaviour, especially in behaviourally complex tasks (Floresco, 2015).

In contrast with the effects of inhibiting activity in the NAcC, inhibition of the NAcS during acquisition did not modify choice behaviour, nor did it influence performance. This was surprising, as several proposed functions of the NAcS seem relevant to performance on the rGT,
including the region’s role in suppressing “goal-irrelevant” behaviour or responding for less desirable options (Floresco, 2015). Given the choice between a four pellet and a one pellet reward, animals reliably choose the four-pellet option (95%); NAcS inactivation somewhat reduces this preference (to 90%), suggesting that the region plays a role in directing behaviour towards the most desirable options (Stopper & Floresco, 2011). This function of the NAcS may be less relevant to behaviour or difficult to detect during acquisition of the task for several reasons. For one, choice is distributed across four options and there is high between-subject variability in preference for these options; marginal shifts in preference are difficult to detect under these conditions. Furthermore, animals are still learning the task and by definition have not arrived at the optimal pattern of choice. Deviations from optimal behaviour may therefore be undetectable at this stage in training. The NAcS also may dampen the influence of irrelevant stimuli on behaviour, such as cues that do not predict reward (Ambroggi et al., 2011). The rGT may not examine this behaviour, as it is not explicitly cued, and each option returns reward, even as there are variations in the amount of reward returned. Repeating the current work with the more recently developed Cued rGT (CrGT) might clarify this region’s role in the suppression of responding to irrelevant cues. The CrGT pairs salient cues with reward delivery, and the magnitude of the cues grows disproportionately larger as the rewards become larger and more uncertain. Behaviour on this task is modified relative to the rGT, with animals demonstrating a putatively cue-driven bias towards the larger but ultimately more disadvantageous options. In theory, inactivation of the NAcS may thus impair performance on the CrGT, given the prominence of stimuli that designed with an intentional mismatch between the long-term advantageousness of the option and the salience of the audiovisual cues paired with it. While not ‘goal-irrelevant’ per se, the cues on the CrGT are roughly inversely proportional to how
advantageous each option they are paired with is and are also sufficient to bias choice towards these options (Barrus & Winstanley, 2016), making them at least superficially similar to the cues in Ambroggi et al. that are not predictive of reward and yet able to promote responding.

In sum, the results described here clarify the roles of the NAcC and NAcS across both learning and acquisition of the rGT. The NAcC contributes to learning, possibly via directing attention to the salient losses associated with some options, but not learned behaviour. In contrast, the NAcS does not appear to contribute to either learning or well-trained behaviour. Repeating this work with the CrGT could elucidate the manner in which salient win-related cues modify the influence of each of these regions on decision making.
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<th>Sessions 6-10</th>
<th>Session 11-15</th>
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<td>13.38 +/-1.93</td>
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<tr>
<td>P1</td>
<td>22.83 +/-2.66*</td>
<td>27.06 +/-2.75**</td>
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.05 = *, .01 = **, .001= ***

Table 5-1: Effects of NAcC inhibition on choice behaviour in acquisition. Data shown are mean ± SEM.
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.05 = *, .01 = **, .001= ***

Table 5-2: Effects of NAcS inhibition on choice behaviour in acquisition. Data shown are mean ± SEM.
### Table 5-3: Effects of CNO administration on choice behaviour at baseline.

Data shown are mean ± SEM.

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<td>Premature response (%)</td>
<td>24.79 +/- 2.96</td>
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<td>22.71 +/- 2.45</td>
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<tr>
<td>Choice latency</td>
<td>1.90 +/- 0.14</td>
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<td><strong>1.39 +/- 0.07</strong></td>
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<td>Collection latency</td>
<td>1.46 +/- 0.10</td>
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<tr>
<td>Trials</td>
<td>92.60 +/- 5.99</td>
<td>92.00 +/- 5.55</td>
<td>108.17 +/- 5.39</td>
<td>108.53 +/- 4.47</td>
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<tr>
<td>Omissions</td>
<td>1.14 +/- 0.33</td>
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<td>0.70 +/- 0.21</td>
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<tr>
<td><strong>NAcS CNO</strong></td>
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<tr>
<td>Premature response (%)</td>
<td>18.02 +/- 2.45</td>
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<td>Choice latency</td>
<td>2.43 +/- 0.18</td>
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<td>Collection latency</td>
<td>1.57 +/- 0.12</td>
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<td>Trials</td>
<td>78.30 +/- 5.38</td>
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<td><strong>116.14 +/- 4.39</strong></td>
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<td>Omissions</td>
<td>3.24 +/- 0.74</td>
<td>2.04 +/- 0.49</td>
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<td><strong>Saline</strong></td>
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<tr>
<td>Premature response (%)</td>
<td>17.98 +/- 0.15</td>
<td>15.46 +/- 1.26</td>
<td>18.14 +/- 1.69</td>
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<td>Choice latency</td>
<td>2.55 +/- 0.15</td>
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<td>2.31 +/- 0.48</td>
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<td>2.68 +/- 0.54</td>
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.05 = *, .01 = **, .001= ***
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<td>NAcC</td>
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<td>Premature response (%)</td>
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<td>1.17+/−0.10</td>
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<tr>
<td>Premature response (%)</td>
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<td>18.61+/−3.53</td>
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<tr>
<td>Choice latency</td>
<td>1.30+/−0.14</td>
<td>1.59+/−0.17</td>
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<td>1.68+/−0.20</td>
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<tr>
<td>Collection latency</td>
<td>1.09+/−0.10</td>
<td>1.16+/−0.09</td>
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<td>Trials</td>
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<td>1.90+/−0.77</td>
<td>1.36+/−0.47</td>
<td>1.19+/−0.40</td>
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Table 5-4: Effects of CNO administration on non-choice behaviour at baseline. Data shown are mean ± SEM.
Figure 5-1: Location and range of acceptable DREADDs expression. Two animals from the NAcS group and one from the NAcC group were excluded because expression was poor or incomplete. Final group sizes were as follows: NAcS = 22; NAcC = 23. Scale bar = 1mm.
Figure 5-2 Choice of each option over sessions 1-20 in the NAcC group. Inhibition of the NAcC lead to slightly elevated choice of P2 (panel A), the most optimal option, and lower choice of P1 (panel B), relative to controls. In both cases, this effect remitted shortly after animals stopped receiving CNO injections. There was a transient yet significant increase in choice of P4 in the NAcC inhibited animals (panel D), but as this difference emerged well after CNO administration stopped, it may not have resulted from drug treatment. There was no significant effect on choice of P3 (panel C). Data shown are mean ± SEM. * denotes p < 0.05.
Figure 5-3 Choice of each option during baseline CNO administration in both the NAcC and NAcS groups. CNO administration did not affect choice behaviour in either group. Data shown are mean ± SEM.
Chapter 6: Inactivation of the orbitofrontal cortex reduces irrational choice on a rodent betting task

6.1 Introduction

Human decision making is often guided by cognitive biases rather than pure rationality (Tversky et al., 1974). Reliance on such heuristics, while theoretically appropriate from an evolutionary perspective, can nevertheless promote suboptimal choice in the modern world, and may play a significant role in the development and maintenance of conditions such as gambling disorder (GD) (Toneatto et al., 1997; Lesieur et al., 1987; Steenbergh et al., 2002; Raylu et al., 2004). Elucidating the neurobiological mechanisms underlying these biases could therefore deepen our understanding of the decision-making process in general, and also contribute to more effective treatments for disorders like GD or even substance addiction where aberrant decision making is prominent.

Animal models are a valuable investigative tool with which to explore cognitive biases. The rat Betting Task (rBT) was designed to capture one commonly-observed decision-making bias, namely subjects’ decreased willingness to accept a gamble as the amount at stake increases, even when the relative utility of the two bets remains constant (Trepel et al., 2005). For example, most people will choose to gamble when offered a choice between $1 or a 50:50 chance of $2 or nothing, yet switch their preference when the guaranteed reward increases to $100. This has been described as escalation of commitment, or risk aversion, and is mathematically inconsistent (Kahneman & Tversky, 1979). In the rBT, animals likewise choose between two options with equivalent expected value, one of which yields a guaranteed reward while the other results in 50% chance of double that bet size or nothing. A subset of “wager sensitive” animals shift their choice preference away from the uncertain option as the bet size grows, reminiscent of a risk averse choice preference. While a wager sensitive choice preference does not result in
acquisition of fewer pellets, and is thus not economically disadvantageous, it is arguably irrational in that this re-evaluation of uncertain outcomes does not correspond to any actual shift in the value of the options offered. In this regard, wager sensitivity resembles the biased behaviour often demonstrated by human gamblers. Erroneous subjective beliefs about uncertain outcomes can take many forms, from craps players throwing dice with more force in an attempt to roll a higher number (Henslin, 1967), to the classic Gambler’s Fallacy whereby individuals believe that if some random event occurs more frequently than normal over a period of time (i.e. a coin flip resulting in many consecutive “heads”), it will occur less frequently in the future.

In past work using the rBT, the degree of wager sensitivity was negatively correlated with D_{2/3} receptor density in the dorsal striatum (Cocker et al., 2012), suggesting potential overlap between irrational choice under uncertainty and alterations in dopamine signaling comparable to that underlying vulnerability to chemical dependency. Given that impairments in frontal functioning are thought to critically contribute to addiction disorders and aberrations in complex decision-making, we theorised that further investigation into the neural correlates of wager sensitive behavior should focus on the prefrontal cortex. While the role of frontal regions in mediating this behaviour are unknown given the limited investigation into this particular bias, findings from other animal models of high-order cognitive processing point to the ventromedial prefrontal cortex and the orbitofrontal cortex as candidate regions of interest.

The infralimbic cortex (IL), situated in the ventromedial prefrontal cortex, has been strongly implicated in behavioural inhibition; silencing this region increases premature responding, a measure of impulsive action (Chudasama et al., 2003; Hosking et al., 2015), impairs the extinction of conditioned fear (Quirk et al., 2006) and promotes drug-seeking in extinction-reinstatement assays (Peters et al., 2008). Inactivation of the IL also induces risk-
averse choice in the rat Gambling Task (rGT), a rodent paradigm loosely analogous to the Iowa Gambling Task in which optimal choice requires the integration of probabilistic gains and losses of varying magnitudes. Collectively, these data indicate that maladaptive choice may arise through lack of reflection as a result of IL inactivation. Hence, inactivation of the IL may precipitate or enhance wager-sensitivity. In contrast, silencing activity in the more dorsomedial prelimbic region (PrL) results in the opposite effect to IL inactivation, reducing relapse-vulnerable behaviours in extinction-reinstatement models (e.g. Peters et al., 2008). In addition to the core cognitive functions of attention (Birrell & Brown, 2000) and working memory (Granon et al., 1994; Dalley et al., 2004), the PrL also appears to play an important role in contingency detection, a function that may be involved in mediating choice on the rBT, given the changes in bet size that take place across each session. While its precise functions are subject to debate, the orbitofrontal cortex (OFC) is implicated in numerous cognitive functions related to affective decision-making, particularly with respect to cognitive flexibility as well as the somewhat-related processes of updating and representing subjective value (Stalnaker et al., 2015; Kringelbach, 2005). This putative function of the OFC may well be critical to choice bias within the rBT, as wager sensitive animals appear to be re-evaluating the relative value of certain and uncertain options as a function of bet size.

Given the functions already ascribed to frontal regions, examining the contribution of the OFC, PrL or IL to non-normative choice patterns may provide valuable insight into the cognitive processes underlying wager sensitivity in addition to our primary goal of determining the neural substrate of this decision-making bias. We therefore sought to determine whether wager sensitive patterns of decision-making on the rBT were likely mediated by processes relevant to representation of subjective value, contingency detection, or behavioural disinhibition, through
comparison of the effects of inactivating the OFC, PrL or IL. We aimed to capitalize on the pronounced individual differences in baseline choice preference typically observed using the rBT in interpretation of our results: similar effects on behaviour across wager-sensitive and – insensitive rats independent of bet size would indicate a general change in outcome evaluation as a result of frontal inactivation that would not be specific to the wager-sensitive bias. In contrast, selective effects of a regional inactivation in one subgroup, such that wager-sensitivity was either selectively induced in previously insensitive animals or selectively attenuated in wager-sensitive rats, would highlight a specific role for that region in mediating wager-sensitivity.

6.2 Additional methods

6.2.1 Subjects

Subjects were 32 male Long-Evans rats (Charles Rivers Laboratories, St. Constant, Quebec, Canada) weighing 250-300g upon arrival at the animal facility.

6.2.2 The rodent Betting Task (rBT)

The rBT and associated training protocols have been extensively discussed in previous publications (Cocker et al., 2012; Tremblay et al., 2014). A task schematic is provided in Figure 6-1. Before the task began, levers were permanently designated as “safe” or “uncertain,” and these designations were counterbalanced across subjects. All trials were initiated via a nosepoke response into the illuminated food tray. Following such a response, the tray light was extinguished and one, two, or three lights within the five-holed array were illuminated in holes 2, 3, or 4. The number of lights presented was equivalent to the number of sucrose pellets available on the safe lever (one, two or three) for that trial. Rats were required to make a nosepoke response at each illuminated aperture to turn off the light inside. Once all the stimulus lights had been turned off, the levers were extended into the chamber. A response on the “safe” lever led to
the guaranteed delivery of the number of pellets wagered, whereas a response on the “uncertain” lever yielded a 50% chance of double the available safe reward or nothing. The expected utility of both options was therefore equal, and there was no net advantage in choosing one lever over the other. On rewarded trials, the designated number of pellets was dispensed into the food tray. Regardless of whether reward was delivered, the tray light was illuminated after a response had been made on one of the two levers, and a response at the food tray initiated the next trial. Failure to respond on either lever within 10 s led to the trial being scored as a choice omission. Similarly, failure to respond at all illuminated apertures within 10 s resulted in the trial being scored as a hole omission. Both errors of omission were immediately punished by a 5 s time-out period. During such time-outs, the house light illuminated the chamber and no reward could be earned or trials initiated. Following the time-out period, the tray light was illuminated, and the animal could commence another trial.

Each session consisted of 12 blocks of 10 trials each. The bet size remained constant within each block but varied between blocks in a pseudorandom fashion that ensured four blocks of each bet size within a session, and not more than 2 consecutive blocks of the same bet size. The first four trials of each block were forced choice, such that only the safe (2 trials) or uncertain (2 trials) lever was presented in random order to ensure the animal sampled from both options throughout the session and was familiar with the current contingency in play. Sessions lasted until all 120 trials had been completed, up to a maximum of 30 min. Animals received five daily sessions per week and were tested until a statistically stable pattern of responding was observed across all variables analyzed over three sessions (total sessions to behavioral stability, 21 to 25). All animals were able to complete the 120 trials within the 30 min time limit.

6.2.3 Surgery
Once animals had achieved behavioural stability, they were divided into two equal groups matched for baseline performance, and were implanted with bilateral guide cannulae targeting either the prelimbic cortex (PrL) \( (n=16) \) or the lateral orbitofrontal cortex (lOFC) \( (n=16) \). following stereotaxic coordinates (Seamans, Floresco, & Phillips, 1995; Zeeb et al., 2010b): PrL: anteroposterior (AP) +2.6, medial/lateral (ML) +/-0.7 and dorsal/ventral (DV) -2.6 ; lOFC: AP +3.8, ML +/-2.6, DV -2.9.

6.2.4 Infusions

The infusion procedure was based on previously published methods (Winstanley et al., 2003). Once behavioural stability was re-established, animals were given two mock infusions to habituate them to the infusion procedure. Both mock infusions and infusions were performed on a three day cycle. On day one, animals performed a baseline session; on day two, animals were infused with either drug or vehicle, and day three was a rest day during which animals remained in their home cage.

All animals were infused with both vehicle (0.9% saline) and a GABA agonist cocktail composed of the GABA B agonist Baclofen (Sigma-Aldrich, Oakville, Ontario, Canada) and the GABA A agonist Muscimol (Sigma-Aldrich, Oakville, Ontario, Canada) \( (0.125\mu g \text{ of each in } 0.5 \mu l \text{ saline}) \). Animals with lOFC cannulations received one round of infusions, whereas animals with PrL cannulations received two rounds, the first targeting the PrL and one round targeting the IL. The order in which the drug was administered was counterbalanced across animals. At least one week elapsed between infusions into the PrL and IL.

6.2.5 Drugs

Drugs were purchased from Sigma-Aldrich. Solutions were prepared fresh each day. Baclofen hydrochloride and muscimol hydrobromide were each dissolved separately in 0.9%
saline at a concentration of .5mg/ml, then mixed together in equal parts to produce a final solution with a concentration of .25mg/ml of each drug (as per (Floresco et al., 2006; Zeeb et al., 2010).

6.2.6 Data analysis

All data analyses were performed with SPSS for Mac (Version 20.0.0; IBM). The following variables were analyzed: percent choice of the uncertain lever; lever choice latency; reward collection latency; choice omissions; hole omissions; trials completed. All data were subjected to repeated-measures analysis of variance (ANOVA). Bet size (3 levels, 1-3 sugar pellets) was included in all ANOVAs as a within-subjects factor. For lever choice latency and reward collection latency, the lever chosen (2 levels, safe vs uncertain) was also included. As per our previous report (Cocker et al., 2012), individual rats were classified as wager sensitive or wager insensitive based on their choice of the uncertain lever during the saline infusion. The choice of the uncertain option at each bet size was plotted in order to generate an equation of the form

\[ y = mx + c \]

in which the factor m indicates the gradient of the line i.e. the degree to which choice of the uncertain option changed as a function of increasing bet size. Animals were classified as wager sensitive if this value fell more than one standard deviation below a theoretical zero. This distinction was used as a between subjects factor (group, 2 levels) in all ANOVAs. When comparing baseline performance over experimental epochs, the group assignments remained stable over all but 3 animals in the OFC inactivation group that switched from being wager-sensitive to wager-insensitive following OFC cannulation. The reason for this behavioural
change is unknown, and was not associated with variation in cannulae placement or any accompanying gross histological changes indicative of OFC damage. Any percentage variables were arcsine transformed to minimize artificial ceiling effects.

Prior to experimental manipulation, each cohort of rats was trained to behavioural stability, as demonstrated by a lack of significant session and bet size x session effects in a repeated-measures ANOVA with session (5 levels, sessions 1-5) as a within-subjects factor. Behavioural effects of each regional inactivation was determined by ANOVA with drug (2 levels, saline, GABA agonist cocktail) as a within-subjects factor. Given the expected difference in baseline choice in wager sensitive and wager insensitive rats, and our a priori assumption that these two groups would differ in their response to manipulations of the frontal cortex, the behavioural impact of inactivating the different frontal regions was also analysed in each group independently.

6.2.7 Histology

Once behavioural testing was completed, animals were anesthetized with inhalable isoflurane and then euthanized via exposure to CO₂. The brains were removed and fixed in 4% paraformaldehyde for at least 24hrs, then frozen and sliced on a cryostat in 50 µm slices. Location of injector tips were plotted onto standard rat brain sections (Paxinos et al., 1998).

6.3 Results

6.3.1 Histology and exclusion criteria

Animals were excluded from the experiment if the location of injector tips was determined to be outside of the region of interest. The location of all acceptable placements are depicted in Figure 6-2. From the cohort assigned to receive IL/PrL infusions, one wager-insensitive animal died as a result of post-surgical complications. During PrL inactivations, three animals (one wager sensitive, two wager insensitive) were excluded from the analysis for
executing an insufficient number of trials (total trials ≤ 5). Following PrL inactivations, three animals died as a result of post-inactivation complications (all wager insensitive) before receiving IL inactivations. Unlike PrL inactivation, no animals from either group had to be excluded for insufficient trials during IL inactivation. Data from 12 animals were therefore included in analysis of PrL inactivations, and 12 for inactivations of the IL. The PrL group was composed of 9 wager-insensitive animals, and 3 wager-sensitive animals. The IL group was composed of 8 wager-insensitive animals, and 4 wager-sensitive animals. For animals assigned to receive IOFC infusions, two animals died as a result of post-surgical complications, one was excluded for completing insufficient trials (total trials ≤ 5), and one was excluded on the basis of incorrect cannulae placement, resulting in a final cohort of 12 animals included in the analysis. The IOFC group was composed of 8 wager-insensitive animals and 4 wager-sensitive animals.

6.3.2 Lateral orbitofrontal cortex inactivation

As per previous reports, individual differences were clearly evident in rats’ choice of the uncertain lever across bet size, leading to the classification of rats as either wager insensitive or wager sensitive following saline infusions (see methods above; bet size x wager sensitivity: $F_{2,20} = 10.72, p = 0.001$). Wager insensitive rats had fairly equivalent rates of responding on both the certain and uncertain lever regardless of bet size ($n = 8$, bet size: $F_{2,14} = 0.76, p = 0.485$). Such indifference is mathematically normative, as the two options are equivalent in terms of expected value at each decision point. In contrast, wager sensitive rats chose the certain lever more often as the bet size increased, a subjective choice preference reminiscent of risk aversion in that willingness to tolerate uncertain outcomes decreased as the size of the reward at stake grew ($n = 4$, bet size: $F_{2,6} = 21.09, p = 0.002$). With the exception that wager insensitive animals made slightly more hole omissions (wager sensitivity: $F_{1,10} = 6.79, p = 0.026$), all other behavioural
measures were comparable in wager sensitive and wager insensitive rats (all Fs ≤ 3.48, p ≥ .099).

When data from all rats were pooled, inactivation of the IOFC increased choice of the uncertain lever in a bet size-dependent manner (drug x bet size: F_{2,20} = 5.39, p = 0.013). However, as shown in figure 6-3, this effect was driven by the behaviour of wager sensitive rats that shifted their preference away from the certain lever at the largest bet size when activity in the IOFC was reduced, somewhat ameliorating the risk-averse response bias evident in this subgroup at baseline (Figure 6-3, panel A; wager sensitive: drug x bet size: F_{2,6} = 6.58, p = 0.031; bet size 1: drug: F_{1,3} = 0.50, p = 0.530; bet size 2: drug: F_{1,3} = 0.08, p = 0.792; bet size 3: drug: F_{1,3} = 14.79, p = 0.031). Further analysis reveals that OFC inactivations lessen the slope of the wager sensitivity function in wager sensitive animals (m-value: saline: -20.20 +/- 5.47, drug: -9.00 +/- 6.58; t(3) = 5.55, p = 0.012). In contrast, inactivation of the IOFC did not significantly alter choice patterns in wager insensitive rats (panel B, wager insensitive: drug x bet size: F_{2,14} = 2.19, p = 0.149), nor was the slope of the wager sensitivity function altered (m-value: saline: -0.38 +/- 7.59, drug: 6.27 +/- 11.80; t(7) = 1.38, p = 0.209).

In addition to these effects on choice, OFC inactivation increased collection latency on safe trials (Table 6-1; drug: F_{1,10} = 6.49, p = 0.034), as well as hole omissions at bet size 3 (drug x bet size: F_{2,20} = 6.07, p = 0.009; bet size 3: drug: F_{1,10} = 10.24, p = 0.010) and decreased the number of trials animals performed (drug: F_{1,10} = 6.93; p = 0.025). Inactivation also slightly increased choice omissions in wager sensitive animals at bet size 3 (drug: F_{1,10} = 5.56, p = 0.040; drug x bet size x wager sensitivity: F_{2,20} = 3.94, p = 0.036; bet size 3: drug: F_{1,10} = 5.46, p = 0.042; drug x wager sensitivity: F_{1,10} = 5.46, p = 0.042). Though these changes achieved
statistical significance, the magnitude of these changes was so small that the functional impact was likely negligible.

6.2.3 Prelimbic inactivation

PrL inactivation did not affect choice behaviour in either the wager sensitive or insensitive animals (Figure 6-3C, D; Drug: $F_{1,10} = 0.86$, $p = 0.375$; drug x bet size: $F_{2,20} = 0.95$, $p = 0.405$; wager sensitive: drug: $F_{1,2} = 1.07$, $p = 0.409$; drug x bet size: $F_{2,4} = 1.69$, $p = 0.294$; wager insensitive: drug: $F_{1,8} = 0.68$; $p = 0.435$; drug x bet size: $F_{2,16} = 0.28$, $p = 0.759$). However PrL inactivations increased choice latency on the risky lever at bet size 1 (Table 6-2; drug: $F_{1,10} = 6.31$, $p = 0.036$; drug x bet size: $F_{2,16} = 2.8$, $p = 0.091$); an effect driven by increases in choice latency at bet size 1 (Table 6-2; bet size 1, saline v drug: $F_{1,10} = 2.75$, $p = 0.017$). Additionally, inactivation increased collection latency on the safe lever (drug: $F_{1,8} = 5.58$, $p = 0.046$) though there was no interaction with bet size (drug x bet size: $F_{2,16} = 2.55$, $p = 0.109$) all other behavioural measures were unaffected by PrL inactivation (all $F$ values $\leq 4.94$, $p \geq 0.057$).

6.2.4 Infralimbic inactivation

Similarly, inactivation of the IL did not affect choice behaviour in either the wager sensitive or the wager insensitive groups (Figure 6-3E Table 6-3, F; Drug: $F_{1,10} = 0.11$, $p = 0.750$; drug x bet size: $F_{2,20} = 0.23$, $p = 0.796$; wager sensitive: drug: $F_{1,3} = 0.11$, $p = 0.757$; drug x bet size: $F_{2,6} = 3.26$, $p = 0.110$; wager insensitive: drug: $F_{1,7} = 0.00$, $p = 0.966$; drug x bet size: $F_{2,14} = 0.24$, $p = 0.79$), though it had other minor behavioural effects. Specifically, IL inactivation increased choice latency on the safe lever (drug x bet size: $F_{2,18} = 12.49$, $p < 0.001$; drug x bet size x wager sensitivity: $F_{2,18} = 6.76$, $p = 0.006$), an effect driven by an increase at bet size 1 (bet size 1, saline v drug: $F_{1,11} = 9.13$, $p = 0.012$) and confined to wager sensitive animals (drug: $F_{1,3} = 1.11$, $p = 0.369$; drug x bet size: $F_{2,6} = 11.71$, $p = 0.008$; bet size 1: $F_{1,3} = 10.27$, $p = 0.049$). IL
inactivation did not affect any other behavioural measures (all F values ≤ 2.74, p ≥ 0.132). Baseline choice preference for the certain option increased in the wager-sensitive group between PrL inactivation and IL inactivation (PrL: Bet size 1: 53.28+/−7.82; IL: Bet size 1: 80.53+/−5.28).

While an addition animal was included in the IL analysis, the presence of this animal does not account for the shift in baseline preference (IL: Bet size 1: Included: 80.53+/−5.28; Excluded: 80.98+/−7.44). Instead, the difference in baseline preference reflects a whole-group shift in favor of certainty as time progressed. While this shift in preference is unexpected, there are two reasons why we did not consider it disqualifying. For one, animals in the PrL/IL cohort did not change group membership once stable choice behaviour was established, with all wager-sensitive animals remaining wager-sensitive for the duration of the experiment and wager insensitive animals remaining wager insensitive. Furthermore, m-value, the key metric of wager sensitivity, did not significantly differ between infusions, indicating that while the intercept of the wager sensitivity function changed over time, the fact of their wager sensitivity did not change (m-value ANOVA: Region being infused: F₁,19 = 2.972, p = .101; Region being infused x wager sensitivity: F₁,19 = 0.064, p = .803). While it is interesting that wager sensitive animals became more uncertainty-averse as the experiment progressed, it might be thought of as a feature of wager sensitivity worthy of future examination, rather than a concern about the reliability of the measure.

6.4 Discussion

Here we show that inactivations of the lOFC significantly ameliorated the mathematically-irrational shift away from the uncertain outcomes as the bet size increased in wager sensitive rats. In contrast, inactivation of neither the Prl nor IL altered choice patterns on the rBT. Such a selective pattern of results indicates that the ability of OFC inactivations to modulate wager-sensitive choice cannot be attributed to lower frontal activity in general.
Instead, our findings suggest that the OFC plays a unique role in mediating this type of decision-making bias, which may inform our understanding of the cognitive processes involved in non-normative choice strategies.

It is worth considering whether the behavioural changes observed during IOFC inactivation were due to off-target diffusion of the baclofen-muscimol cocktail. The area affected by the pharmacological inactivation is estimated to be less than 1 mm$^2$ (Floresco et al., 2006), therefore the relatively large dimensions of the OFC are sufficient to contain this spread. It is nevertheless possible that the most ventral region of the primary motor area was impacted by the inactivation. Although increases in response latencies and omissions were observed following intra-OFC infusions of the GABA agonist cocktail, these were very slight and are not indicative of a general motor impairment triggered by silencing motor cortex. The IOFC is also adjacent to the agranular insular (AI), and a growing body of literature suggests this area may play an important role in gambling-related decision making and addiction (Naqvi et al., 2007; Pushparaj et al., 2012; Clark et al., 2014; Pushparaj et al., 2015). However, inactivations targeting the AI are considerably more caudal and ventral, and result in distinct patterns of behavioural change as compared to inactivations of the IOFC (e.g. St Onge and Floresco, 2010; Pushparaj et al., 2015; vs Zeeb et al., 2015). It is therefore unlikely that the effects observed here arise through substantial inactivation of the AI.

Our interpretation of the effects of IOFC inactivation surmises that an increase in choice of the uncertain option in wager-sensitive animals reflects an amelioration of their wager-sensitive pattern of choice behaviour. Another interpretation that has been proposed is that an increase in choice of the uncertain options is simply regression to the mean. While this is a reasonable concern at first blush, we have several reasons to believe that this cannot explain the
observed effects. For one, animals would have been excluded from our analysis if they did not exhibit statistically stable patterns of responding over the three sessions immediately preceding regional inactivations. However, no animals had to be excluded from our analysis because of session to session variability. Wager sensitivity is a well-conserved pattern of responding across the duration of the experiment, and even if qualities of the wager sensitivity change over time (ie the rate at which animals will select certain options), changes in choice strategy from wager sensitive to wager insensitive (or vice versa) are uncommon. Animals are well trained by the point at which they undergo infusions, having completed between 26-31 session of the rBT. Behavioural stability was checked both before surgery and again before infusions took place. Furthermore, we administered infusions according to a counter-balanced design in order to minimize any possible effects of day-to-day variations in choice. If regression to the mean could explain changes in wager sensitivity, we would expect the counterbalancing to minimize this effect. Finally, the decrease in wager sensitivity was unique to the wager-sensitive IOFC group; we might expect to see similar effects in the wager sensitive PrL and IL groups if this change was due to regression to the mean. This was not observed, and we are fairly confident that the observed decrease in wager sensitivity is an effect of IOFC inactivation and not an accident of statistics.

The IL has been strongly implicated in behavioural disinhibition, or impulsive action (Chudasama et al., 2003). Elevated motor impulsivity has been reliably observed in a number of conditions, such as ADHD, bipolar, gambling and substance use disorders, that are also characterised by impulsive choice (Verdejo-García, Lawrence, & Clark, 2008; Winstanley, Eagle, & Robbins, 2006). Such a decision-making style is exemplified by either selection of smaller-sooner over larger-later rewards using delay discounting methodology, or favoring risky,
economically-disadvantageous options on tasks like the Iowa Gambling Task (Evenden et al., 1996; Bechara et al., 1994). While different aspects of impulsivity do not tend to correlate with each other in small experimental cohorts (Winstanley, 2011; Mitchell et al., 2005; Crean et al., 2002; de Wit, 2009; Winstanley et al., 2004), we recently observed a significant association between increased motor impulsivity and disadvantageous decision making on a rat gambling task in a large healthy population of animals (Barrus et al., 2015). Inactivation of the IL reduces optimal choice on the rGT, further suggesting that similar neurobiological mechanisms are implicated in both impulsive action and maladaptive decision-making under uncertainty (Barrus et al., 2015; Zeeb et al., 2015). Given the decision-making demands in play in the rBT, the IL appeared to be a promising target for investigation. However, IL manipulation did not alter choice on the rBT, indicating that decision-making biases on the rBT may not arise through the same mechanism as motor impulsivity. In support of this conclusion, wager sensitive decisions are not made more rapidly than wager insensitive judgements, further suggesting that increased choice of the guaranteed reward is not made without due consideration, or as a result of behavioural disinhibition. While high motor impulsivity and over-reliance on decision-making heuristics may facilitate addiction disorders (Michalczuk et al., 2011), they may do so via distinct frontostriatal pathways. Indeed, low D_{2/3} receptor binding in the ventral striatum is associated with higher premature responding (Dalley et al., 2007), whereas low levels of D_{2/3} receptor expression in the dorsal striatum is linked to wager sensitivity (Cocker et al., 2012).

While the ventromedial PFC is sometimes investigated as a homologous unit (Buchanan et al., 1994), substantial evidence points to functional dissociation between the IL and PrL (Takagishi et al., 1991; Vertes, 2004). It has been theorised that the PrL is particularly critical for contingency learning; muting the PrL impairs animals’ ability to detect the relationship
between actions and outcomes, facilitating the development of habitual rather than goal-directed strategies (Balleine & Dickinson, 1998). The fact that inactivation of the PrL does not convert wager sensitive patterns of choice into a wager insensitive profile may indicate that indifference to the two options does not represent an automatic, habit-like decision-making strategy. Likewise, the null effects of PrL inactivations across groups indicate that neither wager sensitive nor wager insensitive choice necessarily results from an ongoing PrL-dependent assessment of action-outcome contingencies. The PrL also plays a key role in aspects of attentional processing and working memory (Granon et al., 1994; Muir et al., 1996). The bet size in play is signalled by the number of apertures illuminated, and hence nosepoke responses required, at the start of each trial. A wager insensitive profile could therefore theoretically result from an inability to attend to, or hold on-line, the information signalled by additional cues. Inactivating the PrL also impairs cue-guided behavioral switching (Baker & Ragozzino, 2014). Again, the null effects of PrL inactivation suggest that neither of these cognitive functions are primarily guiding trial-by-trial decision making in well-trained rats, although future studies remain to determine whether this region plays a role in the acquisition of choice preferences.

The fact that neither PrL or IL inactivations ameliorated wager sensitive choice contrasts with the fact that large lesions of the mPFC lead to relative indifference between the smaller-sooner vs larger-later options in a delay-discounting paradigm, as if the rats were no longer sensitive to the shifting contingencies in play across successive blocks of trials (Cardinal et al., 2001). Similar mPFC lesions also lead to suboptimal decision-making on the rGT, as do inactivations of the IL and PrL as noted above (Paine et al., 2013; Zeeb et al., 2015). An important distinction between the rBT and mPFC-dependent paradigms is that both options are matched for expected utility in the former: there is no economic advantage to favoring either
option. The neural circuitry mediating decisions under such circumstances may differ from that recruited when choice must be consistently optimized, through the balancing of costs and benefits, in order to maximize returns. After all, such computation has an energetic cost, and evolutionary pressures would favor more efficient decision-making.

Extrapolating from this line of reasoning, the lack of PrL effects in the rBT further indicates that wager sensitive rats are not computing the objective value of the two options and erroneously determining that the uncertain option yields comparatively less reward as the bet size increases. To phrase this another way, it seems unlikely that wager-sensitive rats are unable to correctly determine that the uncertain option is matched in expected value to the certain outcome. If the wager-sensitive animals really were making errors in contingency detection, we may expect PrL inactivations to lessen or eliminate wager sensitivity. Numerous studies have demonstrated that rats are capable of determining which of multiple options yields the most reward using reinforcement schedules far more subtle and sophisticated than those used here (e.g. Balleine and Dickinson, 1998; Killcross and Coutureau, 2003; Lucantonio et al., 2014; McDannald et al., 2014). While there are numerous functions of the OFC that might be relevant to the observed effects of inactivation, we believe that it is most likely that any bias towards one lever over another arises through differences in perceived, or subjective value. The current demonstration that inactivation of the OFC can ameliorate this bias supports such a conclusion; perhaps more than any other brain region, activity within the OFC has been associated with the manifestation of an individual’s subjective preference for a particular outcome (Kringelbach et al., 2003). Electrophysiological recordings indicate that neurons within this region would selectively fire to a preferred reward, and modulate their firing rate if the animals’ preference changed, e.g. through devaluation (Rolls, 2000, 2006).
However the OFC is not the only brain region to exhibit such properties. Neurons in the basolateral amygdala, for example, also track reward value in an odor discrimination paradigm (Schoenbaum et al., 1998; Schoenbaum et al., 1999). Perhaps critically, lesions to the BLA do not alter the choice pattern of either wager sensitive or insensitive rats (Tremblay et al., 2014). As compared to the BLA, it has been theorised that the OFC may be preferentially involved in the regulation of reward value in response to changing contingencies (e.g. Winstanley et al., 2004; Schoenbaum et al., 2009). Inactivation of the OFC may therefore have prevented wager sensitive rats from revising the representation of the subjective value of the uncertain option as a function of the amount at stake, or in utilizing such subjective value judgments in the decision process, resulting in amelioration of wager sensitive choice. Such an explanation would also explain the null effect of inactivation in wager insensitive animals—these rats do not subjectively re-evaluate the relative value of each option according to bet size, therefore the IOFC may not be critically involved in the decision-making process. If inactivations of the OFC dull the capacity to amend or utilize the subjective value of a reward, animals may be forced to rely on other, more objective forms of reward representation such as the absolute amount of sucrose available. To coin terminology from the somatic marker hypothesis, wager sensitive rats may be following their “gut instinct” that drives them away from the uncertain option as the bet size grows (Damasio, 1994), and this is diminished when activity in the OFC is subdued. This is not dissimilar to the concept of model-based or model-free behaviour, where the OFC is thought to be critical to the ability to use complex models of the task space over more simple strategies than those that arise from pure reinforcement learning (Hampton, Bossaerts, & O’Doherty, 2006). Here, the wager sensitive animals on the rBT may be using a model that devalues the
uncertain option as the bet size increases, and inactivation of the OFC inhibits their ability to draw upon this model, shifting them towards a more wager-insensitive choice strategy.

The question then remains as to whether the OFC is signaling this risk-averse tendency in all rats, but is over-ruled or ignored in wager insensitive animals, or whether the signal does not develop in animals showing this more normative choice pattern. This is not the first paradigm in which individual differences in decision-making at baseline predict whether IOFC inactuations will impact behaviour; similar findings have been obtained using a cued version of the delay-discounting paradigm, in which a visual cue bridged the delay between choice of the larger reward and its delivery (Cardinal et al., 2000). In this task, only animals that ostensibly made use of the cue, and chose the larger delayed reward more frequently than the smaller immediate alternative, exhibited an increase in preference for the smaller-sooner reward following inactivation of the IOFC (Zeeb et al., 2010). With respect to the rBT, low levels of D_{2/3} receptor density in the dorsal striatum are associated with greater wager sensitivity (Cocker et al., 2012). The projections from frontal cortex to the striatum are topographically arranged, with greater innervations from mPFC to ventral striatum, and IOFC to more dorsomedial striatal regions (Berendse et al., 1992; Schilman et al., 2008). As such, it is possible that a wager sensitive decision-making style arises through activity in this OFC-DMS pathway, whereas a more normative strategy operates through an alternative as-yet-unspecified route.

While wager sensitive choice patterns do not disadvantage subjects on the rBT, evidence suggests that an over-reliance on subjective evaluation of reinforcers can be maladaptive in other contexts, particularly with respect to commercial gambling scenarios (see Clark, 2010), but also appropriate management of investments in order to maximize rates of return. Risk aversion has been heavily linked to individuals’ failure to capitalize on investment opportunities (Bajtelsmit &
VanDerhei, 1997). Similar to the current findings, this tendency is reduced in patients with damage to the OFC (Shiv et al., 2005a; Shiv et al., 2005b). The heuristics governing our decision making evolved to deal with a very different set of priorities from those endemic in the modern world. As such, the inability to appropriately evaluate objective and subjective rewards could contribute to a variety of disorders of decision making, and the rBT may be a valuable tool with which to probe such biases.
Figure 6-1: Schematic of the rat betting task (rBT). The rat initiated each trial by making nosepoke response at the illuminated food tray. The tray light was then extinguished, and 1-3 response holes were illuminated, signalling the size of the bet or wager (1-3 sugar pellets). A nosepoke response at an illuminated aperture turned off the light inside it. Once all the aperture lights had been extinguished in this manner, 2 levers were presented to the rat. Selection of the uncertain lever resulted in a 50:50 chance of receiving either double the wager or nothing, whereas selection of the safe lever always lead to delivery of the wager. Adapted from (Cocker et al., 2012).
Figure 6-2: Histological verification of cannulae location. Location of all acceptable orbitofrontal injector tips (A) and prefrontal injector tips (black dots: prelimbic cortex; grey dots: infralimbic cortex) (B) Coordinates are relative to bregma. Plates modified from (Paxinos & Watson, 1998)
Figure 6-3. Inactivating the lateral orbitofrontal cortex (IOFC), but not prelimbic (PrL) or infralimbic (IL) cortices, ameliorated wager sensitive choice on the rBT. The choice behaviour of wager insensitive rats was not affected by inactivation of any frontal region. Data from the IOFC inactivations is shown in panels A (wager sensitive) and B (wager insensitive), PrL inactivations in panels C (wager sensitive) and D (wager insensitive), and IL inactivations in panels E (wager sensitive) and F (wager insensitive). Data shown are mean ± SEM. * denotes p < 0.05.
<table>
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<tr>
<th>Wager Sensitive: Saline</th>
<th>Trials</th>
<th>Betsize</th>
<th>Choice Latency (Safe)</th>
<th>Choice Latency (Risky)</th>
<th>Collection Latency (Safe)</th>
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Table 6-1: Effects of OFC inactivations on non-choice variables in the rBT. Latency data are given in seconds. Data reported are mean ± SEM.
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Table 6-2: Effects of PrL inactivations on non-choice variables in the rBT. Latency data are given in seconds. Data reported are mean ± SEM.
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<td>1.72 ± 0.29</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>2.88 ± 1.52</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>2.06 ± 0.31</td>
<td>2.43 ± 0.55</td>
<td>0.44 ± 0.04</td>
<td>0.57 ± 0.12</td>
<td>3.00 ± 1.92</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1.89 ± 0.22</td>
<td>1.67 ± 0.21</td>
<td>0.42 ± 0.03</td>
<td>0.44 ± 0.06</td>
<td>3.25 ± 2.06</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1.63 ± 0.20</td>
<td>1.49 ± 0.16</td>
<td>0.51 ± 0.10</td>
<td>0.45 ± 0.08</td>
<td>4.13 ± 2.09</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Table 6-3: Effects of IL inactivations on non-choice variables in the rBT. Latency data are given in seconds. Data reported are mean ± SEM.
Chapter 7: General discussion

7.1 Summary of experimental findings

Experiment 1 paired salient cues with risky and disadvantageous choice options in a rodent gambling task, and demonstrated that the addition of these reward-paired cues was sufficient to increase choice of these options at the expense choice of safer, more certain options. The addition of these cues enhanced the role D₃ receptor-mediated signaling played in regulating choice behavior. On the CrGT, D₃ antagonism decreased choice of the risky cue-paired options, while D₃ agonism increased choice of these options. These effects were not observed in the uncued version of the task. In contrast, amphetamine modified choice on the rGT but not the CrGT.

Taken together, these findings suggest that the addition of salient cues modifies the role of DA in this form of cost-benefit decision making. D₃ receptors have been implicated in addiction, and the CrGT may be able to capture decision-making deficits representative of those seen in gambling and other disorders of addiction. Performance on the CrGT may be underpinned by addiction-like neurobiological processes, and drugs that can improve decision-making on this task may have significant clinical benefit in treating the disordered decision making that features prominently in addiction like behaviour.

In experiment two, pharmacological inactivation of the OFC during initial sessions of the CrGT resulted in a significant improvement in choice that was maintained throughout acquisition. Inactivation of the OFC once the CrGT had been learned did not result in a similar shift in choice preference, indicating that the OFC may be most behaviourally relevant when the animal is learning about the contingencies of the task, rather than when it is executing well-
learned behaviour. This result differs from the findings of similar work done with the uncued rGT; when the task does not feature salient, win-paired cues, OFC inactivation during acquisition simply delays the development of the optimal choice strategy, but does not otherwise change choice behaviour (Zeeb, Floresco, & Winstanley, 2010a). The contrast between these results suggests that activity in the OFC is uniquely recruited by the presence of salient cues and is essential to the development of a cue-biased pattern of responding on the CrGT. Inactivation of the BLA at either the start of training or during baseline performance did not have a significant effect of choice behaviour on the CrGT, a result that also contrasts with findings from the rGT, where BLA inactivations delayed acquisition and impaired baseline performance.

The results of experiment 2 suggest that the presence of cues modifies the involvement of both the BLA and the OFC in the development and expression of this form of complex cost-benefit decision making, and that activity of OFC may promote the development of disadvantageous, cue-biased decision making. These findings clarify the neuroanatomical underpinnings of decision making on the CrGT and in the context of salient cues more generally, and suggest that the OFC should be the target of future work to clarify the regional pharmacology and connectivity that drives these effects.

In experiment 3, chemogenetic inhibition of the NAcC during acquisition of the rGT appeared to accelerate development of the optimal choice strategy. NAcC inactivation caused animals to shift their choice towards P2, the most optimal choice, earlier in training, and away from P1, an option with which delivers smaller, more certain rewards and less frequent punishments. In contrast, inhibition of the NAcC made after the task had been learned did not affect choice on the rGT, though it did affect the length of time it took animals to make a choice and collect reward. Inhibition of the NAcS did not affect choice behaviour on the rGT, regardless
of the whether this occurred during acquisition or at baseline. These results suggest that, although well-learned behaviour on the rGT is not critically dependent on either the NAcC or the NAcS, the NAcC is at least partially responsible for the bias towards P1 evident early on in the task, which must be overcome if animals are to truly maximise the reward earned.

In experiment 4, inactivations of the lOFC significantly ameliorated the mathematically-irrational shift away from the uncertain outcomes as the bet size increased in wager sensitive rats performing the rBT. In contrast, inactivation of neither the Prl nor IL altered choice patterns on the task. Such a selective pattern of results indicates that the ability of OFC inactivations to modulate wager-sensitive choice cannot be attributed to lower frontal activity in general. Instead, our findings suggest that the OFC plays a unique role in mediating this type of decision-making bias, which may inform our understanding of the cognitive processes involved in non-normative choice strategies. The OFC is involved in updating and representing subjective value, and it is possible that the pattern of wager sensitivity seen on the rBT reflects animals re-evaluating the subjective value of the uncertain option according to the amount at stake.

In sum, the work presented in this dissertation clarifies the contributions of structural elements of gaming paradigms, gambling-related cognitive biases, and select brain regions and neurotransmitters to gambling-like decision making. Specifically, we’ve demonstrated that salient cues can promote patterns of disadvantageous decision making on a rodent analogue of gambling behaviour, and provides evidence that the addition of these salient cues changes the recruitment of the dopamine system as well as the BLA and OFC relative to uncued tasks. Furthermore, the effects of OFC inactivation on behaviour on both the rBT and CrGT suggests the OFC may be pivotal in promoting subjective choice biases, which are a key component of
GD. Work with the rGT demonstrates a role for the NAcC, but not the NAcS in guiding choice when a task is being learned. Some of the implications of these findings are discussed below.

7.2 Theoretical implications, predictions for future studies

The development of an animal model in which salient cues exert influence over decision making promises to facilitate research into a wide variety of problematic and pathological behaviours in which cues have been shown to promote risky and disadvantageous decision making, such as gambling (Griffiths, 1993; Griffiths & Parke, 2005; Kushner et al., 2007; van Holst et al., 2012) and substance use disorders (Carter & Tiffany, 1999; Childress et al., 1993). The development of the CrGT allows researchers to employ more direct measures than what is possible in human research to examine the regional and pharmacological mediators of cue-biased decision making; such research could facilitate the development of therapeutics for these conditions (Potenza, 2009). Furthermore, the CrGT and derivatives of the model might clarify the cognitive and structural mechanisms by which cues are able to bias decision making.

7.2.1 Mechanisms of influence of salient cues on decision making

As described previously, the CrGT employs sucrose reward, time-out punishments, salient audio and visual cues, and varying probabilistic schedules of reward and punishment. Some exploratory work with variations on the task has demonstrated that animals do not show a choice bias for options with relatively more complex cues if those options offer identical schedules of reward and punishment to those featuring more simple cues (Barrus & Winstanley, 2016). The more complex cue then is not sufficient to drive behaviour in its own right, as would be expected if the cue had intrinsic incentive value. Instead, the cue must be biasing choice through its interactions with one or more of the contingencies of the task, such as the size or frequency of the sucrose reward or the punishment timeouts. Interestingly, the addition of cues
seems to promote choice of the smaller ‘risky’ option P3, rather than P4, despite the fact that P4 offers both more salient cues and larger sucrose pellet reward (Adams et al., 2017). One possible explanation for this phenomenon may be that cues amplify the attractiveness of uncertainty; P3 is the maximally uncertain option on the CrGT, with reinforcement occurring on 50% of selections. Reward-paired cues are capable of evoking DA release in the NAc of humans with a SUD (Volkow et al., 2006) and animals who have been trained to associate them with cocaine administration (Ito, Dalley, Howes, Robbins, & Everitt, 2000), and this DA release is essential to the ability of animals to respond appropriately to these reward-paired cues (Ambroggi et al., 2011). Furthermore, this DA release in response to reward-predictive cues is highest at maximal uncertainty (Fiorillo, Tobler, & Schultz, 2003). Dysfunction in NAc DA may lead to the over-attribution of incentive salience to reward-related cues, increasing attention to and engagement with these cues and promoting maladaptive behaviour (Heinz et al., 2004). Investigating DA release in the NAcC and NacS on the CrGT might clarify the regions roles in cue-mediated behaviour. The functions of the NAcS seem particularly relevant to the CrGT even if inactivations of the region did not modify behaviour on the rGT (Chapter 5), as it appears to inhibit cue-induced responding and approach to non-reward paired cues (Ambroggi et al., 2011; Di Ciano et al., 2008); the NAcS may regulate responding for salient stimuli when this behaviour is not goal directed.

Future work could specifically examine the interaction between cues and uncertainty in promoting risky choice via modifications to the CrGT itself, or examine this putative neuropharmacology through the application of highly specific techniques such as chemogenetic inhibition in transgenic animal models that could isolate the role of NAc DA neurons in CrGT performance.
7.2.2 Behavioural inflexibility and the CrGT

As described in the discussion of experiment 2, performance on the CrGT appears to be more inflexible than performance on the rGT. Reinforcer devaluation is a classic test of whether or not behaviour is goal directed. In theory, if animals attribute some value to sucrose pellets and understand that their behaviour on the task controls the amount of sucrose they can accrue, then changing the value of the sucrose pellets will change their behaviour on the task (Dickinson, 1985). One way of devaluing sucrose pellets is to feed the animals to satiety on those sucrose pellets immediately before they perform the task. Doing so modifies behaviour on the rGT by reducing choice of the most advantageous option (Zeeb & Winstanley, 2013), but it does not modify behaviour on the CrGT (experiment 2). This lack of effect of reward devaluation on CrGT performance suggests that choice on the CrGT is inflexible, rather than purely goal-directed, in that it is difficult to change choice preference via pharmacological or surgical manipulations. Furthermore, the contrast with rGT devaluation indicates that cues are sufficient to cause this shift from goal-directed to inflexible responding. This has implications for behaviours ranging from gambling to substance use, where salient cues have been shown to be behaviourally relevant (Flagel et al., 2009; Grant & Bowling, 2014; Schulze & Jones, 1999; van Holst et al., 2012). If cues promote the development of inflexible patterns of responding, they may play an essential role in the progression of addictive and compulsive behaviour. Future work with the rGT and CrGT should take advantage of this discrepancy between behaviour on each task to examine the processes which may lead to this more rigid, less easily disrupted pattern of choice behaviour. This prospective work could take several approaches, targeting either regions of interest, connectivity between regions of interest, systemic pharmacology or region-specific pharmacology.
One prominent theory that relates the development of addiction to habitual responding characterizes addiction as a transition from deliberate choice to use substances to a habitual or compulsive pattern of behaviour, and that this progression is accompanied by a shift from reliance on the ventral striatum to more dorsally-mediated processes (see Everitt & Robbins, 2005 for review). This habitual responding seems reminiscent of the behavioural inflexibility observed in well-trained animals performing the CrGT. Work with the rGT has so far only targeted the ventral striatum (Experiment 3), and future work targeting the dorsal striatum in both tasks may provide information about the role of the region in CrGT performance, and how this differs as a function of the presence of salient cues. If the development of choice preferences on the CrGT are dependent on dlStr-mediated processes, inactivation of the region may modify performance of the task once it has been acquired. Interestingly, the OFC also projects directly to the dorsolateral striatum (Henk, Wright, & Uylings, 1997). Given the OFC’s demonstrated contributions to choice on the CrGT (Experiment 2) and the prospective role of the DlStr in maintaining habitual behaviour, this connectivity between regions may represent a link in the cortical network that underpins choice behaviour on both the CrGT and rGT.

Work in our lab and others also suggests pharmacological targets for this investigation into the mechanisms of behavioural inflexibility. Administration of 5HT-2C antagonists dose-dependently improved choice on the CrGT, but not the rGT (Adams et al., 2017). The same compound mitigates compulsive behavior (Schepisi, De Carolis, & Nencini, 2013) and improves reversal learning (Boulougouris, Glennon, & Robbins, 2008), suggesting that it promotes behavioural flexibility, a mechanism which may explain its ability to improve choice in the CrGT. The improvement of reversal learning promoted by 5HT2C antagonism can be replicated by direct injections to the OFC (Alsiö et al., 2015) but not other areas of the prefrontal cortex.
The OFC’s contributions to CrGT behaviour may then be 5HT2C dependent, and future work with the task should investigate the pharmacological specificity of the effects reported in experiment 3.

Noradrenergic systems represent another attractive target for investigation into the development and maintenance of inflexible behaviour on the CrGT. While tonic firing of noradrenergic neurons promotes exploration and behavioural flexibility (Aston-Jones & Cohen, 2005), phasic firing is associated with selective attention and exploitation of known environments. Furthermore, this phasic firing can be evoked by salient stimuli (Aston-Jones & Bloom, 1981), suggesting a mechanism by which the cues of the CrGT could encourage the development of a more inflexible choice strategy on the task. In theory, noradrenergic agonism might then improve choice on the CrGT by discouraging rigid adherence to a learned choice strategy, and increase the exploration of the other options.

7.3 Limitations, considerations
A critical limitation of the research conducted for this dissertation is that it was conducted using only male rats. Men demonstrate higher rates of GD than women (Desai & Potenza, 2008), and males are more likely than females to report higher levels of impulsivity, alcohol abuse and dependency, and antisocial personality traits, while females report higher rates of comorbid mood disorders (Desai & Potenza, 2008; Ibanez, Blanco, Moreryra, & Saiz-Ruiz, 2003) and abuse as children (Petry et al., 2005). These authors postulated that GD in males was predominantly driven by impulsivity, while females developed GD as in response to negative affect, and warned that findings about the causal mechanisms of GD found in studies of male populations may not be applicable across sexes. In the IGT, men choose from the advantageous decks more frequently than women, and show differences in activity across the OFC,
dorsolateral prefrontal cortex (dPFC), and right and left hemispheres (see van den Bos, Homberg, & de Visser, 2013 for review). In a maze-based mouse model of the IGT, male rats showed higher choice of the advantageous arms, and showed less day-to-day variation in choice preference than females (van den Bos, Lasthuis, den Heijer, van der Harst, & Spruijt, 2006). Clearly, sex differences in either human populations or animal models cannot be discounted, and future work with these animal paradigms should incorporate subjects of both sexes in order to broaden the applicability of their findings. Our lab has recently begun to use mix-sex designs which incorporate both male and female subjects, which will provide additional information about potential sex differences in task performance and the psychobiological mechanisms that underlie such differences.

Chemogenetic and site-specific pharmacological techniques also allow for precise targeting of the connections between regions, an approach which was not explored in this dissertation. Instead, the experiments described here that employed pharmacological or chemogenetic techniques to inhibit discrete regions of the brain did so serially, inhibiting a single region bilaterally in each subject. While this technique provides valuable information about the contributions of a given region to behaviour, it does not provide information about the relationships between regions, and how regions work together to promote or inhibit behaviour. Future work should target regional connectivity, rather than regions in isolation, as these experiments have done.

7.4 Concluding remarks

In sum, the work here has informed our understanding of the contributions of several prefrontal regions and the NAc to models of animal decision making. The development of the CrGT meaningfully improves upon contemporary knowledge derived from more simplistic
paradigms that may not accurately capture the real-world decision making involved in gambling and substance addiction. These experiments lay the foundation for future work in our laboratory to further characterize regional and circuit-level contributions to performance on these animal models of biased decision making. The CrGT has the promise to enhance our basic understanding of how cues shift behaviour towards more disadvantageous options, knowledge that might have far-reaching implications for all manner of cue-mediated behaviour.
Bibliography


Disorders. In Curr Topics Behav Neurosci (pp. 507–529).


Impulsive Choice Induced in Rats by Lesions of the Nucleus Accumbens Core. Science, 292, 2499–2501.


Dalley, J. W., Laane, K., Theobald, D. E. H., Armstrong, H. C., Corlett, P. R., Chudasama, Y., &


Kruzich, P. J., See, R. E. (2001). Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. Journal of Neuroscience. 6(8), 1255–1264


Specker SM, Carlson GA, Christenson GA, Marcotte M (1995) Impulse control disorders and


Zack, M., Featherstone, R. E., Mathewson, S., Fletcher, P.J. (2014) Chronic exposure to a gambling-like schedule of reward predictive stimuli can promote sensitization to amphetamine in rats. Front Behav Neurosci 8:36.


