

Investigating the influence of risky decision making on dopaminergic reward mechanisms

by

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Abstract

Addiction is a chronic relapsing psychiatric disorder affecting millions worldwide. Despite years of research investigating the etiology and phenomenology of substance abuse, there is no cure. Determining factors which promote the addictive phenotype may help to discover new therapeutics. Several clinical studies have shown addicts demonstrate poor cost/benefit decision making as measured by validated tasks such as the Iowa Gambling Task (IGT), a cognitive deficit maintained during periods of abstinence and associated with relapse risk. However, it is unclear whether disadvantageous choice precedes or is the consequence of drug abuse. Furthermore, dopaminergic signalling, actively recruited by drugs of abuse, has also been implicated in decision-making biases, and may contribute to choice deficits after drug exposure. The experiments here explored the role of disadvantageous choice in addiction susceptibility using rodent analogues of the IGT, the rat gambling task (rGT) and cued rat gambling task (crGT). These paradigms require the animal to choose between four different nose poke options which are associated with sugar wins, probabilities of winning, and timeouts. The crGT also includes salient reward-paired cues to enhance risky decision making. The first two experiments assessed whether baseline risk-preference on the rGT and crGT affected drug seeking as measured by cocaine self-administration, and whether drug exposure affected task performance. The third study examined the influence of task experience on the locomotor response to cocaine and responding for conditioned reinforcement, two dopamine-dependent behavioural assays associated with addiction risk. Basal and cocaine-induced nucleus accumbens dopamine release was also assessed using microdialysis after task training. The final study used chemogenetics to

reduce nucleus accumbens dopamine to investigate the role of dopaminergic tone in choice biases. Our results show poor decision making precedes drug exposure, and is uniquely susceptible to drug-induced cognitive deficits. crGT rats showed greater drug seeking and sensitivity to cocaine-induced choice impairments, a phenotype linked to basal accumbal dopamine efflux. Finally, by reducing accumbens dopamine, animals showed marked reductions in risky choice. These data support the conclusion that poor decision making may serve as a cognitive endophenotype for addiction via aberrant dopaminergic signaling within the mesostriatal network.

Lay Summary

Drug addiction is a pervasive psychiatric condition for which there is no cure. Studies have shown that addicts have poor cost/benefit decision making, which may make them vulnerable to drug seeking and relapse. However, it remains unclear whether risky decision making is the product of drug abuse or a risk factor for addiction. A breadth of data has shown dopamine contributes to both drug seeking and decision-making biases, and may therefore underlie vulnerability to both addiction and drug-induced choice deficits. The work in this thesis explores decision making as a vulnerability factor for addiction, and the role dopamine has in the expression of choice biases. The data here support the conclusion that poor decision making is uniquely worsened by drug exposure, and aberrant dopamine signaling in the brain's key reward network mitigates risky choice. These studies provide a behavioural model and biological targets for future research and potential therapeutics.

Preface

Experiment one (chapter 3, all sections) has been previously published in the manuscript by Ferland, JMN and Winstanley, CA. 2016. “Risk-preferring rats make worse decisions and show increased incubation of craving after cocaine self-administration”. *Addiction Biology*, DOI: 10.1111/adb.12388 (copyright licence #4102750815654). The candidate and Dr. Winstanley were responsible for the study concept and design. The candidate collected animal data, performed data analyses, drafted the manuscript, and assisted with interpretation of results. Dr. Winstanley provided critical revision of the manuscript for important intellectual content and interpretation of results.

Experiments two and three (chapters 4 and 5) have been submitted for publication by Ferland, JMN, Hounjet, C.D., Lindenbach, D, Vonder Haar, C, Adams, W.K., Kaur, S., Phillips, A.G., Winstanley, C.A. (2017). The candidate and Dr. Winstanley were responsible for the study concept and design. The candidate collected animal data, conducted pharmacological challenges, cannulation surgeries, data analyses, assisted with microdialysis, drafted the manuscript, and assisted with interpretation of results. Celine Hounjet conducted behavioural testing and assisted with post-operative care. Dr. David Lindenbach provided methodological input for microdialysis, performed microdialysis, HPLC, and assisted with interpreting microdialysis data. Dr. Cole Vonder Haar performed cannulation surgeries. Dr. Wendy Adams bred animals for the experiment. Sukhbir Kaur assisted with the breeding program and genotyped rats used in experiment three. Dr. Anthony Phillips provided crucial experimental input and interpretation of data. Dr. Winstanley helped design the experiment, provided critical revision of the manuscript for intellectual content and interpretation of results.

Experiment four (chapter 6) is being prepared for publication by Ferland, JMN, Feng, T, Adams, WK, Silveira, MM, Kaur, S., Hathaway, B, Russell, B, Winstanley, CA. (2017). The candidate provided input for experimental design, completed statistical analyses, administered pharmacological manipulations, and prepared the figures and manuscript. Tanya Feng conducted behavioural testing, assisted with pharmacological manipulations, and helped with manuscript preparation. Dr. Wendy Adams helped design the experiment, bred rats, and performed AAV microinfusion surgeries. Mason Silveira performed AAV microinfusion surgeries and assisted with pharmacological manipulations. Sukhbir Kaur assisted with breeding, genotyped the animals, and performed immunohistochemistry. Brett Hathaway and Brittney Russell provided technical support. Dr. Catharine Winstanley helped design the experiment, provided important interpretation of data, and critically revised the manuscript.

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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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List of Abbreviations

ac- anterior commissure

ANOVA- Analysis of variance

BLA- basolateral amygdala

CNO- Clozapine-N-Oxide

Cre- cre-recombinase

CRf- Conditioned reinforcement

CRH- Corticotropin Releasing Hormone

crGT- cued rat gambling task

CS- Conditioned Stimulus

CS+: Conditioned stimulus predictive of reward

CS-: Conditioned stimulus not predictive of reward

DA- Dopamine

dStr- dorsal striatum

DREADD- Designer receptors exclusively activated by designer drugs

FR- fixed ratio

GD- Gambling disorder

HPA- Hypothalamic pituitary axis

HPLC- High-performance liquid chromatography

IGT- Iowa Gambling Task

IOC- Incubation of craving

ITI- inter-trial interval

NA- Noradrenaline

NAc- Nucleus Accumbens

PFC- prefrontal cortex

mPFC- medial prefrontal cortex

OFC- orbitofrontal cortex

RDoC- Research Domain Criteria

rGT- rat gambling task

SEM- Standard error of the mean

SNr- substantia nigra

SUD- Substance Use Disorder

Tg- Transgene

Tg+: Transgene positive

Tg-: Transgene negative

TH- Tyrosine Hydroxylase

VTA- Ventral tegmental area

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Dedication

This thesis is dedicated to those who work to provide outreach for, treat, or research mental illness. Hopefully as we gain a better understanding of these phenomena and their underlying biological mechanisms, they will cease to be perceived as hindrance to society, and receive the care and attention they deserve.

Chapter 1: General Introduction

1.1 An introduction to research on addiction: towards cognitive endophenotypes of substance use disorder

Substance use disorder (SUD) is a chronic, relapsing psychiatric condition for which there is no current cure. In Canada, 1 in 5 individuals will struggle with substance abuse at one point during their lifetime, with the total economic burden of addiction amounting to \$39.8 billion annually (Adlaf, 2005; Rehm et al., 2006). Available pharmacotherapies are designed to reduce the subjective experience of withdrawal symptoms and in turn decrease craving and relapse risk (Carrera et al., 2004). However, despite alleviating symptoms in the first 1-4 weeks of withdrawal, many of these drugs fail to prevent relapse in the long-term (Nestler, 2002), and many patients return to drug use within the first year of abstinence (McLellan et al., 2000). Although withdrawal symptoms are transient, craving can persist for extended periods of time and may instigate relapse. This is particularly true of cocaine, for which withdrawal symptoms are relatively acute and mild (Kreek et al., 2002) yet relapse risk is high (Epstein et al., 2006). In addition to the lack of effective therapeutics, illicit substance use is on the rise worldwide (UNODC, 2015), and drug addiction is still one of the most stigmatized mental health disorders, making it difficult for patients to seek treatment (Patel et al., 2015; Saloner et al., 2017). It is clear newer, more efficacious therapeutics are desperately needed to treat SUD.

Decades of clinical research have attempted to disentangle the phenomenology of the addictive cycle: drug craving, followed by seeking/ consumption, withdrawal, and relapse (Koob

and Volkow, 2010). After considerable work, data led to the rise of two prominent theories of addiction. The first is the negative reinforcement theory, which states it is the discomfort associated with abstinence which drives the addict to return to drug seeking more than the pleasure of the drug itself, a prominent issue in alcohol and opiate addictions (Wise and Bozarth, 1987). However, some drugs of abuse do not produce protracted withdrawal states, such as cocaine (Kreek et al., 2002), and many addicts will relapse even years after withdrawal symptoms have ceased (Epstein et al., 2006). The second, the positive reinforcement hypothesis, dictates addiction is precipitated by the overwhelming rewarding properties of the drug, and it is the “pleasure” produced by drugs which drives compulsive use (Stewart et al., 1984). To contradict this theory, there is a great amount of evidence that shows not only do some drugs not produce a “euphoric” state, such as nicotine (Robinson and Berridge, 1993), but addicts rapidly develop tolerance to the reinforcing effects of the drug, and due to the negative consequences of pursuing it, often have interpersonal, employment, and health issues which counteract the pleasant qualities brought on by a high (Falk et al., 1983).

Despite the limitations of each hypothesis, these proposals did help to define the positive and negative valences associated with drug use. Based on these theories, animal research modelled addiction by focusing on how drug availability results in increased responding for drug (Belin and Deroche-Gamonet, 2012), and if available for extended periods of time, escalation of use (Wee et al., 2007). Many studies also looked at how ‘negative’ reinforcement, such as increased stress, may induce relapse (Koob and Kreek, 2007). These endeavours have pointed to crucial subcortical structures, which may facilitate the acquisition of drug use, such as the ventral striatum, ventral tegmental area (VTA), and amygdala (Koob et al., 1994; Koob and Kreek,

2007). However, these hypotheses and results fail to account for the fact that even after years of abstinence, the addict is still at risk for relapse.

A contemporary hypothesis states it is not purely positive and negative affect which influence drug seeking, but aberrant stimulus-outcome associations that become the insidious drivers of addiction. The “incentive sensitization” theory posits cues and contexts in proximity to drug seeking or taking become imbued with “incentive salience”, or become intrinsically reinforcing (Robinson and Berridge, 2008). Over time, these stimuli recruit similar neurobiological mechanisms as the drug, and eventually dictate behaviour in favour of drug seeking (Robinson and Berridge, 2008). In addition to the standard self-administration studies, clinical and animal research began to investigate the involvement of cue responsivity in subjective craving (Volkow et al., 2006; Moeller et al., 2009; Wang et al., 2012). Cue-mediated drug seeking models such as second-order schedules of reinforcement (Arroyo et al., 1998), and cue-induced reinstatement (Epstein et al., 2006), are commonly used to determine how quickly the reinforcing drug-paired stimuli can maintain operant responding for drug or precipitate relapse. One group found that responding for drug-paired cues sensitizes during protracted withdrawal, and the subject is more prone to “relapse” up to ninety days after the final self-administration session (Grimm et al., 2001; Lu et al., 2004), a phenomenon known as “incubation of craving”. These studies have allowed us to disentangle the acute and chronic effects of addictive substances and cues on mechanisms thought to be inherent in the pathophysiology of addiction (Koob and Volkow, 2010; Belin and Deroche-Gamonet, 2012).

Without wishing to dismiss this monumental research effort prematurely, results from these reports interpret robust drug seeking on a cohort level as being akin to the addicted state, despite the fact it is known that not all those who take drugs, even abuse them, will become

addicted (Belin and Deroche-Gamonet, 2012; Belin et al., 2016). The symptoms of SUD such as craving, habitual seeking, and continued use despite harm implicate complex aberrant neurocircuitry regulating reward processes which skew behaviour in favour of drug seeking (Goldstein et al., 2009a; Koob and Volkow, 2010). Having a greater understanding as to which factors contribute to individual variation in addiction risk and are exploited by drug abuse is essential for the development of new treatments and requires further exploration (Belin and Deroche-Gamonet, 2012).

Several factors are associated with addiction risk across the lifespan, including genetic predisposition (Schumann et al., 2011; Stringer et al., 2016), gene/environment interactions (Vink, 2016), abuse (Marcenko et al., 2000), socioeconomic status (Patel et al., 2015), sex (Becker, 2016), and pre-existing mental health conditions (Kirst et al., 2014). Unfortunately, these statistics paint a troubling picture: based on sex and poverty alone, approximately 10-50% of the population is at risk for developing an addiction (Group, 2016). Delineating specific risk factors at the intersection between the influences listed above, and are readily affected by drug seeking, may help to re-frame our understanding of addiction. An initiative by the National Institutes of Mental Health has proposed a new framework for the conceptualization of mental health conditions incorporating such factors: the Research Domain Criteria approach (RDoC; (Insel et al., 2010)). RDoC looks to behavioural, neuroanatomical, and genetic factors that transcend categorical definitions of psychiatric disease to find “domains” which contribute to the expression of psychopathologies. The RDoC approach takes a more comprehensive view of addiction, and investigates more complex perturbations in behaviour and neurocircuitry which may facilitate addiction risk.

With the RDoC framework in mind, there has been a significant research effort to explore the involvement of impairments in higher-order cognitive processes in the behavioural shift from acute to chronic, habitual drug use (Belin et al., 2008; Economidou et al., 2009; Everitt and Robbins, 2013). These studies work under the assumption that neural substrates affected by addiction also underlie cognitive faculties, such as behavioural inhibition and decision making, and symptomatology in one domain may represent vulnerability in the other. For example, poor impulse control has been noted across a variety of substance dependent populations (Jentsch and Taylor, 1999; Moeller et al., 2001; Belin et al., 2016) and has been associated with compulsive use and relapse sensitivity (Dalley et al., 2007; Economidou et al., 2009). The biological contributors to each behaviour include aberrant activity in top-down and bottom-up neuronal networks, indicating both conditions are likely the result of similar neuronal signalling (Dalley et al., 2008; Dalley and Robbins, 2017). However, animal work has shown that, while behavioural disinhibition may be predictive of greater self-administration, this cognitive deficit is only transiently exacerbated or ameliorated after drug exposure (Winstanley et al., 2009; Caprioli et al., 2013), suggesting high impulsivity may facilitate the acquisition of drug seeking but not necessarily the continuation of compulsive use.

On the other hand, maladaptive decision making appears to be central to the phenomenology of addiction, with the addict choosing to pursue the drug despite the associated negative consequences including loss of employment, difficulty with relationships, and damage to health (Bechara, 2005). The perception that it is merely a matter of willpower to “just say no to drugs” leads to heavy stigmatization of the condition (Patel et al., 2015), and frames societal viewing of addicts as deserving of retribution rather than help (Boyarsky et al., 2002). However, if viewed as a neurobiological condition, impoverished decision making may present a unique

and measurable symptom and potential therapeutic target. In fact, many substance abusers demonstrate impaired decision-making performance (Rogers et al., 1999; Bolla et al., 2003; Bechara, 2005; Verdejo-Garcia and Bechara, 2009) including those who use cocaine (Verdejo-Garcia et al., 2007; Stevens et al., 2013), methamphetamine (Gonzalez et al., 2007; Wang et al., 2013), heroin (Verdejo-Garcia et al., 2007), marijuana (Bolla et al., 2005), alcohol (Bechara et al., 2001; Goudriaan et al., 2007), and polysubstance abusers (Grant et al., 2000). Furthermore, poor choice behaviour persists during drug withdrawal, predicts treatment failure, and addiction severity (Bechara et al., 2001; Wang et al., 2012; Stevens et al., 2013), implying that this cognitive deficit critically contributes to the maintenance of the addicted state (Bechara, 2005). Various attributes of decision making also appear to be biased in addiction. For example, one study found methamphetamine abuse was associated with decreased loss anticipation but greater sensitivity to loss outcomes (Bischoff-Grethe et al., 2017), and recent work has shown those with opioid dependence are impaired on a cognitive flexibility task (Liang et al., 2017), a behavioural attribute essential for adapting choice in the face of changing utility. Similar decision-making impairments have been seen in those with gambling disorder (GD), a behavioural addiction in which compulsive gambling is inherently maintained and modulated by disordered choice (Linnet et al., 2011; Clark et al., 2013; Brevers et al., 2016). These data indicate maladaptive decision making may not simply result from any neurotoxic effects associated with drug abuse, but may also precede SUD, biasing crucial reward systems in favour of high risk, high reward outcomes.

Although multiple factors contribute to the manifestation of addiction, recognising a prominent role for disordered decision making may identify new treatment approaches (Bechara, 2003; Bechara, 2005). However, the nature of the relationship between poor decision making

and SUD is difficult to determine from clinical data, in which myriad environmental and circumstantial factors may predominate. Understanding whether poor decision making is a symptom or cause of addiction needs to be explored.

1.2 Modelling cost/benefit decision making in the rat

The operationalization of decision making presents a challenging problem for researchers due to its inherently complex nature. At face value, decision making is the simple choice between options presented, often based on likelihood of outcome. It has an internal driver, a goal, and actions to achieve that goal. However, the contingencies involved are often varied, and tap into different aspects of value and action. For example, you are on a road trip, hungry, and have recently finished your available food in the car. Coming up on a town, you have the option to stop and eat at a restaurant, or continue to the next town for other opportunities. Although the decision seems simple, it incorporates several facets of decision making including intrinsic value of food (how hungry you are, the last time you ate), subjective value of the options (do you want to consume what is available), reward history (whether you have eaten at similar restaurants and enjoyed the product), availability (is the restaurant open, what types of restaurants are there), how long to the next town (delay to reward), and so on. However, despite the complicated nature of decision making, choices in general have long term associations between the option and its contingencies, or the costs and benefits, by which the organism weighs in the presence of internal states and the outcome. This theoretical framework acts as the foundation of basic *cost/benefit decision making*. This conceptualization works under the assumption that choice is based on psychological processes which are subserved by common neuroanatomical and neurochemical networks which converge to drive the subjects' behaviour (Vartanian and

Mandel, 2011), and allows us to design experimental protocols and interrogate neural circuitry implicated in choice.

In human research, cost/benefit decision making is often measured by the subject's choice between a likely option versus an uncertain one. One assay is the prominently used Iowa Gambling Task (IGT), purposefully designed to simulate “real world” decision making in which all choices can lead to both beneficial and detrimental outcomes (Bechara et al., 1994; Bechara et al., 1999a). In this test, subjects pick cards from four decks to accumulate points. The optimal strategy is to choose cards from the two advantageous decks associated with small immediate gains but also low and infrequent penalties. Persistent selection from the two disadvantageous decks leads to large, immediate gains, but heavy losses in the long-term, and clearly represents a maladaptive, risky strategy. This task incorporates several attributes of decision making, such as reward valuation, reward history, and behavioural flexibility thought to be inherently compromised in psychiatric disorders such as addiction (Bechara and Damasio, 2002; Bechara, 2003; Bechara, 2005; Fellows and Farah, 2005). However, addicted individuals who present at the clinic have months, sometimes years, of chronic drug experience, making it difficult to determine whether suboptimal choice is the cause or consequence of habitual drug use. Animal models provide a means to determine behavioural contributions to addiction susceptibility as well as the neural substrates contributing to decision making and psychopathologies (Potenza, 2009).

There have been several preclinical tasks designed to model decision making in a variety of species, but for the purposes of this review, focus will be placed on tasks for the rat. Most decision-making paradigms take place within operant chambers in which environment and manipulanda can be tightly controlled to present the animal with different stimuli that prompt

behaviour. Upon responding to levers or nose-poke holes, rats can be taught actions lead to specific outcomes, such as reward (sugar pellets, sucrose solution) or punishment (shock). Some decision-making tasks are relatively simple two-option paradigms, which have the animals choose between a certain, conservative outcome (i.e. 100% delivery of one sugar pellet), or the uncertain, often termed “risky” option, where the reward value is higher (delivery of 4 pellets) but is associated with a lower likelihood of reinforcement (i.e. 50% probability of winning; (St Onge and Floresco, 2010)), or even punishment if the trial is not rewarded (e.g. shock; (Simon et al., 2011)). Data from these studies have shown animals decide between different probabilistic outcomes, are sensitive to changes in returns, and show manipulation of key neurotransmitter systems are linked to choice biases (Mitchell et al., 2011; Simon et al., 2011; St Onge et al., 2012; Stopper et al., 2013). But these tasks depend on a variety of factors including dynamic changes across session (i.e. probability of winning changes in trial blocks) which the animal must track. Therefore, experimental manipulations may produce differences in sensitivity to reward or acknowledgment of changes in contingency. Furthermore, such tasks have two available options, resulting in the animal being described as mostly conservative or risky, a prescriptive dichotomy which may over-simplify what shifts in behaviour represent (Bhatia and Loomes, 2017). Finally, although the negative outcomes in these studies do influence behaviour, omission of reward and shock are not necessarily analogous to the monetary loss seen on human tasks such as the IGT, and may have limited predictive validity.

The above tasks provide valuable information regarding the neural processes which contribute to choice behaviour, but may lack “behavioural resolution” when it comes to exploring the influence of risk preference on addiction susceptibility. Since 2006, four groups have published rodent variants of the IGT using mazes and operant boxes in attempts to model

decision making seen in humans (de Visser et al., 2011). Each task structure differs in training length, session duration, number of options available, and negative outcome associated with a loss. Such differentiations allow the researcher to probe decision making during distinct types of choice, such as learning the task's contingencies (the "exploration" phase of the task) or how manipulations influence baseline choice strategies (the "exploitation" phase; (Daw et al., 2006)). One model trains rats over 10 sessions to enter four arms associated with sugar wins or quinine (bitter) pellet losses on a probabilistic reward schedule (van den Bos et al., 2014). This paradigm conserves several attributes of the IGT, including similar reward/loss ratios and number of options available. However, the return of quinine pellets can be argued not as a loss, but merely a less valued win. In fact, some animals are known to consume the quinine pellets despite not being appetitive (de Visser et al., 2011), which may be the product of food restriction. Another version utilizes omission of reward to impose "loss" (Pais-Vieira et al., 2007). In contrast to the IGT, the expected utility of the "safe" and "risky" options are similar on this task, meaning this model more closely examines sensitivity to reward probabilities rather than loss itself.

To address these issues, the Winstanley laboratory developed another rodent analogue of the IGT, the rat gambling task (rGT), which uses timeouts as punishment (Zeeb et al., 2009). On this task, rats have 30 minutes to maximize sugar wins by nose-poking into four illuminated response holes. These holes are loosely analogous to the decks of cards in the IGT, and range in amount of reward won (1-4 sugar pellets), probability of winning (0.4-0.9), and length of timeout if the trial is lost (5-40 seconds). Due to limited session length and food restriction, the timeouts prevent the hungry animal from playing subsequent trials, diminishing the total number of sugar pellets earned in a session. Animals readily learn the contingencies of this task and most subjects acquire a decision-making pattern which capitalizes on the most advantageous option (Zeeb et

al., 2009), behaviour that is remarkably similar to that seen in healthy control subjects (Bechara et al., 1994; Fellows and Farah, 2005). Furthermore, manipulations of neurocircuitry including lesions of the orbitofrontal cortex (OFC) and basolateral amygdala (BLA) impact rate of acquisition and expression of optimal choice, respectively (Zeeb and Winstanley, 2011), and mediate updates in value (Zeeb and Winstanley, 2013), not dissimilar to previous reports from the IGT (Bechara et al., 1999a; Fellows and Farah, 2005). Another benefit of the rGT is the inclusion of other cognitive measures, such as motor impulsivity, as determined by the rat's inability to withhold a nose-poke response in the 5 second inter-trial-interval (ITI) prior to the presentation of the four choice options (Robbins, 2002). Therefore, the rGT is a powerful tool to potentially assess cost/benefit decision making and other factors influencing choice behaviour including motivation and impulsivity.

There are also considerable individual differences in preference for the “optimal” and “risky” options of the task (Barrus et al., 2015a), making this paradigm particularly useful for investigating decision making as a cognitive endophenotype for addiction. As in the IGT (Bechara et al., 1999a), the degree to which an animal demonstrates advantageous choice can be calculated as the overall choice score, or the sum of the advantageous options ($P1 + P2$) minus the disadvantageous ($P3 + P4$). A rat with a net positive score is therefore considered to be an “optimal” chooser, whereas animals with negative scores are “risk-preferring”. Interestingly, greater risk-preference is associated with heightened motor impulsivity (Barrus et al., 2015a), two risk factors thought to be important for the acquisition and maintenance of addiction (Bechara, 2005; Winstanley, 2011; Belin et al., 2016). Using the naturally occurring decision-making phenotypes of the rGT, we can investigate how individual differences in risk-preference influence responding to drugs of abuse, and are affected by drug self-administration.

In addition to examining naturally occurring biases in risk sensitivity, it is also prudent to explore whether enhanced risky decision making may influence responding for drugs of abuse. In the case of GD, salient audiovisual cues used in certain electronic games have been known to encourage play, influence perception of wins, and skew decision making (Clarke, 2005; Dixon et al., 2010; Clark et al., 2013; Harrigan et al., 2015). Interestingly, GD is highly comorbid with alcohol and substance abuse (Petry et al., 2005), and evidence suggests increased responding for cues is associated with addiction risk (Flagel et al., 2009; Robinson and Flagel, 2009; Flagel et al., 2010). However, whether maladaptive choice could be facilitated by reward-paired stimuli was unknown. To investigate this possibility, our lab developed the cued rat gambling task (crGT) in which the reward contingencies are identical to the original rGT, but wins are accompanied by salient light/tone cues which increase in variability and complexity with the size of the win. For example, if an animal chooses P1, the most conservative option, and wins, one sugar pellet will be delivered with a single tone and flashing light. In contrast, if the animal selects P4, the most disadvantageous, and wins, it is presented with four sugar pellets, variable flashing lights at the food tray and nose-poke array, and a sequence of tones. The addition of these salient stimuli significantly increased selection of P3, the most uncertain option of the task (0.5 probability of winning), while simultaneously decreasing choice of P2 (Barrus and Winstanley, 2016). Investigating whether crGT training influences sensitivity to drugs of abuse like cocaine, and potential cognitive deficits that result from drug abuse, would provide significant insight as to how choice biases brought on by external forces influence addiction risk.

Recent animal work has also raised the intriguing idea that *repeatedly* engaging with uncertainty itself sensitises the dopaminergic response in a similar way to drugs of abuse (see section 1.3 for review), making the individual more vulnerable to the locomotor and reinforcing

effects of psychostimulants (Bradberry and Roth, 1989; Sadikot and Parent, 1990; Chen et al., 1996; Borgland et al., 2004; Singer et al., 2012; Zack et al., 2014). Results indicate animals exposed to daily training sessions under variable ratio or probabilistic schedules of reinforcement, compared to more certain outcomes, exhibited significantly potentiated ambulatory responses to amphetamine and locomotor sensitization (Singer et al., 2012; Zack et al., 2014). These findings suggest that repeated risky choice may contribute to a sensitized response to psychostimulants and cues.

On the other hand, uncertain reward delivery has also been known to increase the attribution of incentive salience to reward-paired and adjacent stimuli (Robinson et al., 2014). Repeated exposure to a conditioned stimulus (CS) that is associated with a 50% chance of reward delivery (i.e. maximal uncertainty) can increase animals' willingness to approach that CS (Gottlieb, 2004; Anselme, 2015; Cartoni et al., 2015), a behaviour known as "sign-tracking" that is thought integral to incentive sensitization (as discussed above (Robinson and Berridge, 2000; Robinson et al., 2015)). Therefore, selection of the rGT and crGT's risky options may not affect sensitivity to reward, but instead, responding for cues, which may facilitate operant responding for drugs. A simple behavioural paradigm, conditioned reinforcement (CRf), can be used as a test to determine whether a subject is more likely to respond for a cue previously associated with reward (Fanselow and Wassum, 2015). Investigating the role of decision making in addiction susceptibility may point to key neurobiological substrates which subserve reward sensitivity, cue responsivity, and addiction.

1.3 Overview of mesocorticolimbic circuitry, and its involvement in decision making and addiction

A plethora of animal and clinical work has found several neurochemical contributors to the expression of addiction, including the opioid system, thought to mediate the reinforcing effects of drugs (Fields and Margolis, 2015) as well as the distinct agitation during withdrawal (Chavkin and Koob, 2016), serotonin which mitigates responding to the rewarding properties of drugs and associated cues (Cunningham et al., 2011; Anastasio et al., 2014), and glutamatergic signaling implicated in aberrant learning and neuroplasticity after drug exposure (Pierce and Wolf, 2013). Although each of these systems plays a marked role in the addictive phenotype, perhaps the most explored area in addiction neurocircuitry is potentiated dopaminergic activity within the mesocorticolimbic pathway (Koob and Volkow, 2010), which will be the focus of this dissertation. This pathway involves dopaminergic projections from the VTA, a nucleus within the midbrain which synthesizes dopamine (DA), to forebrain structures involved in motivation and reward processing (Morales and Margolis, 2017). DA is synthesized from l-tyrosine to l-DOPA by tyrosine hydroxylase (TH), and subsequently converted into DA by the enzyme aromatic-l-amino-acid decarboxylase (Morales and Margolis, 2017). After production, DA is stored in vesicles until it is released into the synapse. Afferents from the VTA innervate several cerebrum structures involved in reward, affect, and executive function, and DA serves as a neuromodulator of cellular activity at these sites (Morales and Margolis, 2017). There are two major profiles for DA release, tonic and phasic efflux. Tonic release results from spontaneous cell firing and is thought to act as a “baseline” for DA (Goto et al., 2007). In contrast, phasic release occurs after DA neurons “burst fire”, and is often recruited in the presence of rewarding or unexpected outcomes to highlight important or salient events against the backdrop of basal

DA tone (Grace, 2000; Goto et al., 2007; Schultz, 2016b; Morales and Margolis, 2017).

Together, tonic and phasic release regulate the organism's response to salient, rewarding, and aversive outcomes (Cardozo Pinto and Lammel, 2017).

DA acts as the endogenous ligand for a diverse G-protein coupled receptor family, including five subtypes within two major subfamilies- the D₁-like receptors (D₁ and D₅), and the D₂-like family (D₂, D₃, and D₄; (Keeler et al., 2014; Morales and Margolis, 2017)). Generally speaking, activation of the D₁-like family increases postsynaptic cell excitability whereas the D₂ family does the opposite. The expression of these receptors throughout the prefrontal cortex (PFC), dorsal (dStr) and ventral striatum (including the nucleus accumbens; NAc), amygdala, and hippocampus enables DA to mitigate regional responsivity to salient events, and influences activity within top-down and bottom-up networks (Beaulieu and Gainetdinov, 2011), situating it as a key modulator of cognitive and reward-related behaviours. Indeed, there has been a myriad of work to support DA plays a prominent role in disordered cognition including poor impulse control, effortful decision making, and working memory (Cools, 2016). Further investigating the influence of DA activity in choice biases may help to illuminate how this neurotransmitter simultaneously mediates reward sensitivity and cost/benefit decision making.

It is now understood that the majority of drugs of abuse, while acting through a variety of individual mechanisms, directly or indirectly increases VTA DA output, hijacking learning mechanisms and behaviours to promote drug seeking (Robinson and Berridge, 2000; Koob and Volkow, 2010). Work has shown both acute and compulsive drug self-administration elicit phasic DA release first within the NAc, and then in the dStr, suggesting DA is actively involved in biasing motor output in favour of habitual responding for drugs (Willuhn et al., 2012). Furthermore, DA within the ventral striatum facilitates context-outcome associations as

measured by conditioned place preference (Schilwein et al., 1998) and cue-induced reinstatement (Weiss et al., 2001). Sub-regions in the PFC and amygdala are also integral in the development of compulsive drug seeking and relapse. One study found modulation of DA D₃ receptor activity within the BLA attenuated drug seeking under a second-order schedule of reinforcement (Di Ciano, 2008), a paradigm which uses reward-paired cues and intermittent drug delivery to induce robust operant responding akin to addiction (Arroyo et al., 1998). DA activity within the prefrontal cortex and connections to the NAc enhances cue-induced reinstatement (McGlinchey et al., 2016). Interestingly, DA is neither consistently hyper- or hypo-active in addiction, but both. Many studies have shown that active drug abusers demonstrate a *hyper-active* DA response to drugs and cues (Goldstein et al., 2009b; Volkow et al., 2009; Volkow et al., 2010), but in periods of abstinence, these systems become *hypo-active* (Volkow et al., 1996), fostering a “reward deficient” state which robustly responds to drugs of abuse (Blum et al., 2011b; Blum et al., 2011a; Blum et al., 2012a). Areas like the PFC have been found to be hypoactive in abstinent drug abusers, potentially via chronic DA release (Volkow et al., 2009; Chen et al., 2013). This results in both aberrant connectivity to downstream subcortical structures in favour of drug seeking (Ma et al., 2014; McGlinchey et al., 2016) but also limited control over key inhibitory processes which may limit negative behaviours (Dalley et al., 2011; Belin et al., 2016).

In decision making, DA's role is quite complex. One study in humans found reduction of tyrosine by administration of a branched-chained amino acids cocktail increased disadvantageous choice on the IGT (Sevy et al., 2006). On the rGT, acute amphetamine administration impaired choice, whereas a D₂-receptor antagonist improved decision making, suggesting both efflux and receptor activity have discrete control over choice preference (Zeeb et al., 2009). In contrast, modulating D₃ receptor activity mediated risky choice on the crGT, but amphetamine

administration did not significantly impact decision making (Barrus and Winstanley, 2016). From these results, one might interpret that DA has a greater influence on cue-mediated behaviour versus standard cost/benefit decision making. However, work using the probabilistic discounting paradigm found chronic upregulation of DA by amphetamine increased uncertain choice (Floresco and Whelan, 2009) and modulating DA receptor activity within the PFC to BLA/NAc pathway and BLA mediated choice biases (Larkin et al., 2016; Jenni et al., 2017). These data implicate DA in risk-preference, but more work is required to elucidate the specific role it may play in rGT performance.

At a glance, the common mesocorticolimbic substrates underlying decision making and drug seeking seem exceptionally complex, involving both subcortical systems of reward as well as prefrontal modulation of behaviour (Koob and Volkow, 2010). However, several lesion and pharmacological studies point to target regions of interest. A clear first choice would be subregions within the PFC. The PFC receives a myriad of subcortical inputs and uses these signals to withhold or execute a response (Orsini et al., 2015), determine the value of rewards and associated stimuli (Bechara et al., 2001; Stalnaker et al., 2009), and attend to context to guide behaviour (Moorman and Aston-Jones, 2015). Most studies have implicated the OFC as being important for updating reward contingencies and associated behaviours if the outcome is improved, diminished, or reversed (Lucantonio et al., 2012). Lesions or temporary inactivations of the OFC produce impairments in reversal learning and reward devaluation but not acquisition of the original contingencies (Stalnaker et al., 2006). Drugs of abuse have similar effects: repeated researcher- or self-administered cocaine results in intransigent OFC activity, preventing reversal learning and reward devaluation (Stalnaker et al., 2006; Stalnaker et al., 2009).

The medial PFC (mPFC) has also been implicated in drug seeking, as enhanced mPFC activation increases response to drug-related cues after withdrawal (Ma et al., 2014). Lesion and pharmacological studies indicate that the mPFC and OFC help to optimize decision making performance, albeit only modestly (St Onge and Floresco, 2010; St Onge et al., 2012; Zeeb and Winstanley, 2013; Zeeb et al., 2015). OFC lesions made prior to task experience delayed acquisition of the most adaptive strategy on the rGT, whereas lesions made at baseline had no impact on choice (Zeeb and Winstanley, 2011). Temporary inactivations of the mPFC impaired decision making when reward outcomes increased or decreased across the session (St Onge and Floresco, 2010), but had only a mild effect on rGT performance in which contingencies remain stable (Zeeb et al., 2015). Though these data do not rule out involvement of the PFC, they do indicate a stronger subcortical component may drive the maintenance of risk-prone behaviour.

Perhaps the most tempting target is the NAc, known to be integral in stimulus-outcome behaviours (Bissonette and Roesch, 2015), self-administration (Koob and Volkow, 2010), and incubation of craving (Lee et al., 2013; Ma et al., 2014). However, the NAc's involvement in risky decision making remains unclear; inactivation of the NAc decreased risky choice on a probabilistic discounting task (Stopper and Floresco, 2011), while our lab has found NAc lesions did not affect rGT performance at baseline (unpublished observations), nor did inactivations impact task acquisition (Barrus et al., in preparation). However, other studies have found NAc neurons demonstrate differential DA release and cell firing whether the animal prefers risky or safe options (Sugam et al., 2012; Sugam et al., 2014) and are essential for encoding cue-outcome associations (Chang et al., 2012; Hart et al., 2015). Dopaminergic activity within the NAc is very responsive to changes in reward (Hart et al., 2015), largely contributes to the expression of psychostimulant sensitization (Mayfield et al., 1992; De Vries et al., 1998), CRf (Beninger and

Ranaldi, 1992; Wolterink et al., 1993), and has been shown to mitigate probabilistic decision making (Stopper et al., 2013). These results indicate DA activity within the NAc is likely an important neural locus of decision-making biases and requires further exploration.

Although previous pioneering studies into the neurobiology of decision making have indicated a role for the neural substrates discussed above, most of these data were collected using lesions and pharmacological manipulations, which lack specificity. Lesions are prone to compensatory mechanisms, and pharmacological methods are limited by the pharmacokinetic and receptor selectivity of the compounds used. Targeting subregions or pathways involved in choice via pharmacology involves invasive intracerebral microinfusions, which can result in considerable tissue damage and thus are not ideal for chronic drug challenges (Greenshaw, 1998). Furthermore, most of the above manipulations are acute, and long-term alterations in neurobiological systems may have different effects on the expression of behaviour. With the advent of chemogenetic technology, or Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), researchers can administer minimally intrusive systemic ligands which up- or down-regulate cell activity only in neurons expressing the DREADD protein (Zhu and Roth, 2015). Combined with transgenic animals which express cre-recombinase in TH containing cells, we are now able to transfect DA neurons and their projections, and selectively modulate DA release within the mesocorticolimbic network using systemic drug challenges (Witten et al., 2011). Using this technique, not only will we be able to inhibit dopaminergic firing throughout an rGT/crGT session, but we also will be able to chronically regulate dopamine efflux which may have greater influences on behaviour compared to acute manipulations.

In sum, there is a plethora of data to suggest common neurobiological substrates contributing to poor cost/benefit decision making and addiction. We hypothesize that the

propensity to seek risk may not only contribute to the development of an addiction but, due to reliance on the same brain mechanisms, be uniquely affected by drug use. A better understanding of the neurobiological basis underlying risky choice on rat gambling tasks may lead to the identification of novel treatments for addiction that can improve decision making and prevent impairments in judgement caused by drug use. Given the weight of evidence suggesting a strong role for aberrant DA signaling in both risky decision making and the response to addictive drugs, the following experiments therefore aim to explore the role of DA signaling in mediating both maladaptive choice and potential drug-induced cognitive sequelae. We posit risk-preferring animals have a naturally sensitive dopaminergic system, making them more susceptible to the reinforcing properties of the large, uncertain rewards on the rGT, the salient cues of the crGT, and potentially drugs of abuse.

1.4 Measures of the rGT, crGT, and experimental objectives

Using the rGT and crGT allows researchers to investigate perturbations in cost/benefit decision making akin to that seen in humans. Each 30-minute session consists of self-initiated trials during which animals choose among four distinct reward options: two low risk, low reward outcomes, associated with few sugar pellets but higher probabilities of winning, and two high risk, high reward options, where sugar returns are greater but selection of these options will ultimately result in fewer pellets earned due to costly timeouts. Prior to the presentation of the choice options, the rat must also withhold responding during a 5 second inter-trial interval (ITI). If the animal responds during this period, the trial is measured as an impulsive, “premature” response, and is punished with a 5 second time out.

In addition to these central measures of cognition, the gambling tasks also measure important variables of motivation. Omitted responses, or trials in which the animal does not make a choice, measures whether the animal is motivated to make a response. The number of trials completed are also a good indication of how willing the animal is to play the game, although lower trial counts may also be reflective of greater risky choice due to lengthy time outs preventing the animal from continuing. The task also measures the latency to make a choice, which may represent deliberation, and if longer choice latencies are accompanied by greater omissions, may reflect deficits in motivation. Reward collection latencies are also measured, and can be used as an additional variable to determine reward valuation.

Finally, in addition to the resolution given by analysis of the individual choice options, we use the animals' score variable to determine whether that rat is inherently optimally choosing or risk-preferring, allowing us to explore whether baseline risk-preference influences responding for drugs of abuse. Changes in score may reflect overall shifts in risk tolerance brought on by experimental manipulations, and when used in conjunction with changes in individual choices, give the data greater depth of what changes in choice represent.

The four experiments within this dissertation, which have been published, submitted, or are being prepared for peer-reviewed academic journals, aim to delineate the relationship between addiction susceptibility and decision making. They are as follows:

Experiment 1 (chapter 3) investigates the impact baseline risk-preference on the rGT has on the acquisition of cocaine self-administration. It also examines rGT performance during the concomitant self-administration period to determine whether cocaine exposure affects decision making. rGT performance is also measured during a 30-day withdrawal period. Finally, using the incubation of craving paradigm of relapse, we explore whether risky choice affects relapse

susceptibility. This experiment allowed us to determine whether individual differences in risk-preference impacted sensitivity to drugs of abuse, and explored how drug exposure affected cognitive performance.

Experiment 2 (chapter 4) examines the influence of cue-biased decision making on cocaine self-administration. Using a similar approach as experiment 1, rats were trained on the crGT and underwent concurrent behavioural and self-administration sessions to determine whether enhanced risky choice influenced cocaine seeking, and whether drug exposure produced impairments in decision making. A subgroup of rats was placed through 30 days of forced abstinence to determine the effects of withdrawal on crGT performance. These data speak to gaps in the literature exploring whether sensitivity to reward-paired stimuli simultaneously affect choice and addiction vulnerability.

Experiment 3 (chapter 5) assesses whether choice preference or rGT/crGT experience sensitizes the DA system as determined by cocaine-induced locomotor activity. This study also explored whether risk-preference or task experience influenced responding for reward-paired cues as measured by CRF. Lastly, to probe the potential involvement of DA within the NAc, we performed microdialysis to explore basal and cocaine-induced DA release in task experienced animals. To supplement previous research using basic reinforcement paradigms, this study examines how repeated exposure to the uncertain options of the tasks affect the DA response to cocaine.

Experiment 4 (chapter 6) explores the role of DA efflux within the NAc in decision making. Using DREADDs, we acutely downregulated NAc DA release prior to rGT and crGT performance. Then, using a chronic dosing regimen, NAc DA was suppressed for four weeks to investigate if chronically blunted DA tone influenced sensitivity to risky choice. Rats were then

exposed to a final, concurrent amphetamine challenge to determine whether blunted NAc DA affected psychostimulant induced cognitive deficits. Although previous work has indicated an integral role for DA in addiction and decision making, this study focuses on how manipulations in dopaminergic tone influences choice biases and response to psychostimulants.

Chapter 2: General Methods

2.1 Subjects

Subjects used for all experiments were either male Long-Evans rats obtained from an outbred facility (experiments 1 and 2; Charles River Laboratories, St. Constant, Quebec, Canada) or transgenic animals on a Long Evans background bred in-house (experiment 3 and 4). Rats bred in-house were obtained from Charles River Laboratories and the Rat Resource and Research Centre (RRRC, Columbia, MO) as part of a breeding program for transgenic rats that express cre recombinase (Cre) in neurons that contain tyrosine hydroxylase (TH; Long Evans – Tg (TH-Cre) 2.1 Deis, RRRC # 00659). Animals weighed 275-300 g, were adults (post-natal day 90) at the start of the experiment, and were food restricted for 85% of their free-feeding weight (maintained on 14 g rat chow daily). Water was available *ad libitum*. Rats were pair- or trio-housed in a climate controlled colony room maintained at 21° C on a reverse 12-hr light-dark schedule (lights off at 8 am). Testing and housing were in accordance with the Canadian Council of Animal Care and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia.

2.2 Behavioural apparatus

Behavioural testing for the rGT was conducted in standard 5-hole operant chambers enclosed within ventilated sound-attenuating cabinets (figures 2.1A & B; Med Associates, Inc., Vermont, USA). One wall of each chamber consists of an array of 5 response holes, 4 of which

were used during the rGT. The food magazine, positioned 2 cm above the bar floor located opposite to the response holes, was attached to an external food dispenser equipped to deliver sucrose pellets (45 mg, Bioserv, New Jersey, USA) to the magazine. A light stimulus was situated at the back of each response hole as well as within the food magazine. Nose-poke responses into these apertures were detected by a horizontal infrared beam. Chambers could be illuminated by a houselight, and were controlled by software written in Med PC by CAW running on an IBM compatible computer.

Self-administration boxes were identical to those described above but were fitted with an infusion apparatus consisting of a variable rate infusion pump (MedAssociates, Inc., Vermont, USA), a 10 ml plastic syringe used to administer drug or vehicle, PE/PVC tubing (Instech Solomon, Plymouth Meeting, PA, USA) connected to a 22 gauge single-channel plastic swivel (Instech Solomon), and a 40 cm spring-covered tubing connector assembly (Plastics One, Roanoke, VA, USA).

2.3 Habituation and pre-task training

Animals were first habituated to the operant chambers over two daily 30 min sessions during which sucrose pellets were placed in response holes and food magazine. Animals were then trained to nose-poke on a modified version of the five-choice serial reaction time task, where the light stimulus located at the back of response hole 1, 2, 4, or 5 would be illuminated. The spatial location of the stimulus light varied pseudo randomly between trials. Response into the illuminated hole would deliver a sucrose pellet followed by the onset of the next trial for a maximum of 100 trials over a 30 min session. Animals were trained to respond to a light

stimulus lasting 10 sec until $\geq 80\%$ trials were correctly detected and $\leq 20\%$ trials omitted. Rats were then trained on a forced-choice version of the rGT or crGT for seven sessions before moving on to the full free-choice task. This ensured all animals had equal experience with the reinforcement contingencies, and aimed to prevent simple biases toward a particular hole from developing.

2.4 The Rat Gambling Tasks

For a schematic of the tasks, see figure 2.2. Methods are based on previous experiments completed in the lab (Zeeb et al., 2009; Barrus and Winstanley, 2016). During a 30 min behavioural session, trials were initiated by the animal by nose-poking into the illuminated food magazine. Upon responding, the magazine light was extinguished and followed by a 5 s inter-trial interval, after which nose-poke apertures 1, 2, 4, & 5 were illuminated and remained lit for 10 s. Responses into the holes during the 10 s presentation would result in either delivery of a sugar reward or a punishment time-out period during which the chosen aperture's light flashed at 0.5 Hz. Each hole is associated with a different amount of reward (1 - 4 sugar pellets), length of penalty time-out (5 - 40 s), and probability of winning a reward over punishment (0.9 - 0.4). The crGT was identical to the rGT but included salient light/tone cues paired with wins on the task (see table 2.1). Consistent choice of the smaller or larger rewards would yield the most sugar pellets either by the frequency of rewards or by the number of sugar pellets gained per response, respectively. However, due to the length of punishment and low probability of winning, choice of larger reward options (P3 and P4) ultimately led to fewer sugar pellets over the course of the session while choice of the smaller options, particularly P2, resulted in the greatest amount of

reward. The locations of choice options were counterbalanced across animals to control for side biases.

Responses made during the ITI were recorded as premature responses, a measure of impulsive action, which resulted in the illumination of the in-house light and a 5 s time-out penalty before being allowed to start a new trial. If a response was not made into one of the 4 holes during the 10 s stimulus presentation, the trial would be registered as an omitted response at which point another trial would begin. Animals received 5-6 daily sessions per week until statistically stable patterns of behaviour across all measures were observed over three sessions. This took approximately 35 sessions to achieve.

2.5 Self-administration

Animals in experiment one and two (chapters 3 and 4) underwent self-administration after behavioural stability was achieved on the rGT or crGT. To minimize task interference, cocaine self-administration was run in separate operant boxes kept in a different room within the facility. Animals were trained to lever press for cocaine hydrochloride (0.75 mg/kg/infusion calculated as the salt and dissolved in sterile 0.9% saline; Medisca Pharmaceuticals, BC, Canada) or saline vehicle over 10 daily 3-hr sessions (Calu et al., 2007). At the start of the self-administration session, two free infusions of solution were given to fill catheters and indicate drug was available. Rats were presented with two levers, one active and one inactive, with an illuminated cue-light situated over the active lever. Using a fixed ratio (FR1) schedule, responses on the active lever would result in a single 4.5 sec infusion in concert with the cue-light flashing (50 Hz) and a 20 kHz tone. Following the infusion, animals would undergo a 40 s time-out during which the cue-light and tone would extinguish but levers would remain extended.

Responses on the active lever during infusions and timeouts were recorded and interpreted as preliminary evidence of cocaine “seeking”. Inactive lever presses, while monitored, had no programmed consequences. Animals were limited to 30 infusions per hour to prevent overdose. Concurrent rGT and crGT sessions were run in the morning followed by afternoon self-administration sessions to ensure any changes in behaviour were not the result of dosing.

2.6 Jugular vein catheterization surgeries

Rats were anaesthetized using 2% isoflurane gas and given analgesic (ketaprofen, 0.5 mg/kg) and local anaesthetic (bupivacaine). Animals were aseptically implanted with catheters constructed of Silastic silicone tubing (Dow Corning via VWR International, Edmonton, AB, Canada) attached to backmount cannulae (Plastics One, Roanoke, VA, USA) into the right jugular vein. Catheters were passed through the skin subcutaneously and were positioned such that the cannulae exited between the shoulder blades. To prevent blockages, catheters were flushed daily with 0.1 mL of 50% heparinized saline. Animals were allowed 5-7 days of recovery after surgery prior to any behavioural test sessions.

2.7 Genotyping

Rats bred in-house used for experiments three and four (chapters 4 and 5) were genotyped to confirm expression of TH and Cre. After weaning, rats were anesthetized and ear notches obtained for PCR analyses. To extract DNA, ear notches were lysed using a buffer solution (50 mM pH 8.0 Tris, 2 mM NaCl, 10 mM EDTA, 1% SDS) and Proteinase K (Invitrogen, part number 25530-015). Extracted DNA was stored at -20°C until PCR was performed. All reactions were performed in 200 µl thin walled PCR tubes with FASTSTART

TAQ DNA POL. DNTPACK reaction mix (Roche, cat #4738357001), primers for Cre (forward: 5'-AGA GTA CAC TGT GGG CAG GA-3'; reverse: 5'-GCA AAC GGA CAG AAG CAT TT-3') or TH (forward: 5'-CGC TTA CCC CGG AAG AAC AA-3'; reverse: 5'-CCA GCA GAG GTA ATG GAA GAG A-3'). Samples were then placed in a thermocycler and underwent standard cycling protocols (95°C 5 mins; then cycled 35 times: 94°C 30 sec, 63°C 30 sec, 72°C 1 min; holding at 72°C for 10 min; infinite holding at 4°C) and subsequently run on an agarose gel at 90V for 40 min to verify genotypic expression.

2.8 Statistical Analyses

Statistical analyses were completed using SPSS Statistics 24.0 (IBM) or SYstat 12.0 (Chicago, IL, USA) software. As per previous reports, the following crGT and rGT variables were analysed: score $((P1+P2) - (P3+P4))$, percent choice of each option (number of option chosen/ total number of choices x 100), percentage of premature responses (number of premature responses/ total number of trials initiated x 100), sum of omitted responses, sum of trials completed, and average latencies to choose an option and collect reward. Variables that were expressed as a percentage were subjected to an arcsine transformation in order to limit the effect of an artificially imposed ceiling (i.e. 100%) (McDonald, 2009). A statistically stable baseline was determined by a repeated-measures analysis of variance (ANOVA) across data from three consecutive sessions where session and session x choice interactions were not significant. Data from individual animals would be excluded if the rat completed fewer than 20 trials in a session.

For all analyses, if sphericity was violated as determined by Mauchley's test, a Hyundfelt correction would be applied, and corrected p-values' degrees of freedom rounded to the next integer. Results were deemed to be significant if p-values were less than or equal to an alpha of

0.05. Analyses yielding a p-value between 0.05 and 0.07 were reported as trend. Significant effects would be followed up with post-hoc one-way ANOVA or t-tests.

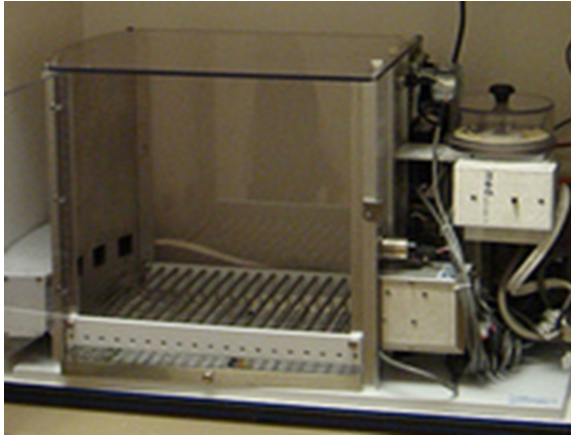
2.9 Table

Option	Cue duration	Auditory cues	Visual cues	Variable?
P1	2s	1 tone 2 tones, in sequence	Flash H1, 2.5Hz, 2s	No
P2	2s	1 s each 3 tones, in sequence	Flash H4, 2.5 Hz, 2s Flash H5, 5 Hz, 1s; Flash	No
P3	2s	0.2 s each 6 tones, in sequence	H2, H3, H4, 5Hz, 1s Flash H2, 5Hz, 1s; Flash H1	Yes, 2 patterns
P4	2s	0.2 s each	H2, H3, H4, H5, 5Hz, 1s	Yes, 4 patterns

Table 2-1 Auditory and visual cues used on the crGT.

2.10 Figures

A.



B.



Figure 2-1 A standard 5-hole operant box.

A) Side view of modular chamber, with response holes visible on the left and food magazine on the right. B) The five-hole stimulus array. Only the outermost holes (1, 2, 4, and 5) were used for rGT and crGT testing.

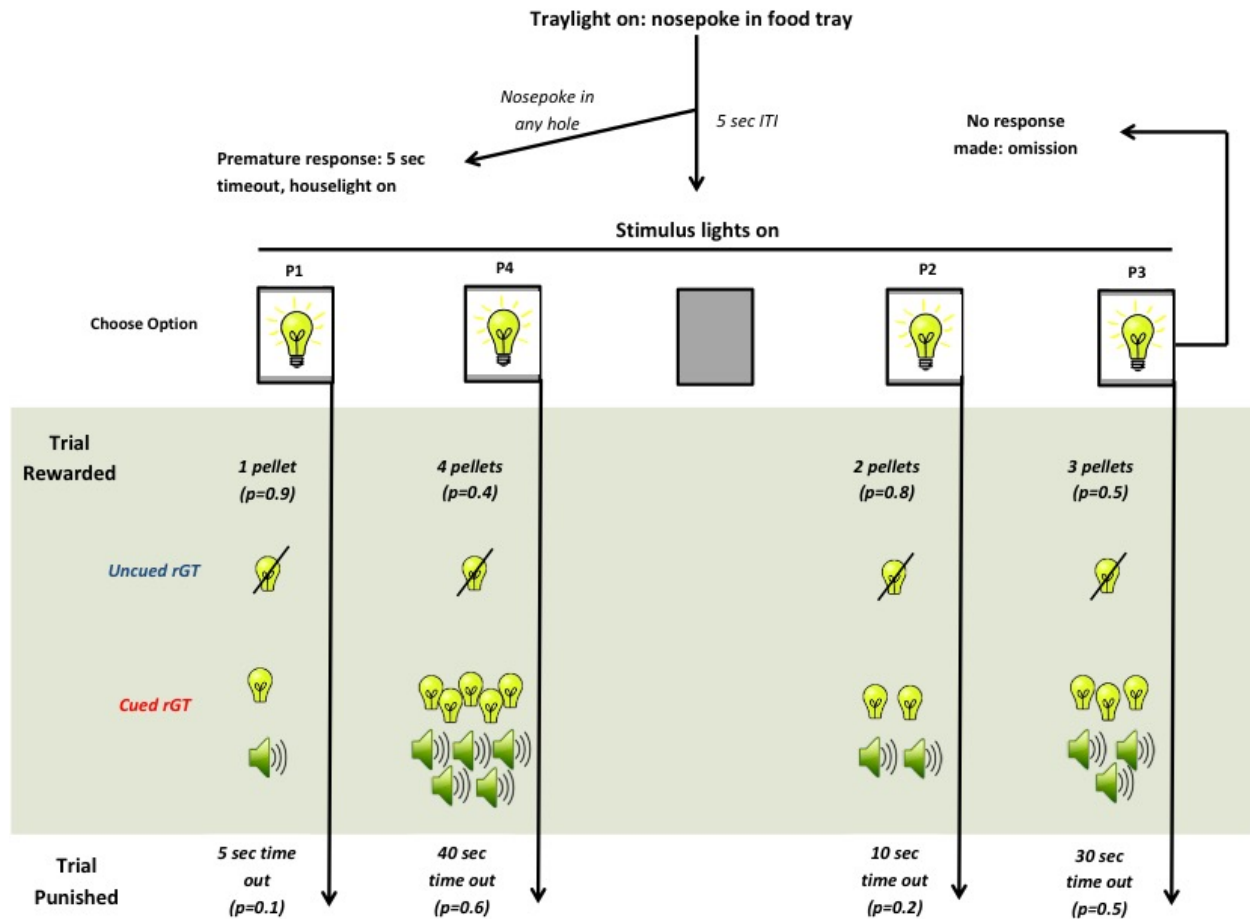


Figure 2-2 Schematic diagram showing the trial structure of the rGT and crGT.

The task began with illumination of the tray light. A nose-poke response in the food tray extinguished the tray light and initiated a new trial. After an intertrial interval (ITI) of 5 seconds, four stimulus lights were turned on in holes 1, 2, 4 and 5, and the animal was required to respond in one of these holes within 10 s. 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 for each option). If the animal was rewarded, the stimulus lights were extinguished and the animal received the corresponding number of pellets in the now-illuminated food tray (crGT rats also received concurrent win-paired audiovisual stimuli). 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 0.5 Hz 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-second timeout during which the house light was turned on (schematic based upon Zeeb *et al.* 2009).

Chapter 3: Risk-preferring rats make worse decisions and show increased incubation of craving after cocaine self-administration.

3.1 Introduction

At the moment of relapse, arguably the most clinically significant point in the addiction cycle, the value of drug use outweighs the benefits of sobriety. As such, maladaptive decision making may be considered central to the phenomenology of SUD (Goldstein and Volkow, 2002; Duka et al., 2011). Indeed, dependent populations perform poorly on laboratory-based cost/benefit decision making tasks (Rogers et al., 1999; Bolla et al., 2003; Bechara, 2005; Verdejo-Garcia and Bechara, 2009). One of the most well-established of such paradigms is the Iowa Gambling Task (IGT), purposefully designed to simulate “real world” decision making in which all choices can lead to both beneficial and detrimental outcomes (Bechara et al., 1994; Bechara et al., 1999b). In this test, subjects pick cards from four decks to accumulate points. The optimal strategy is to choose cards from the two advantageous decks associated with small immediate gains but also low and infrequent penalties. Persistent selection from the two disadvantageous decks leads to large immediate gain but heavy losses in the long-term, and clearly represents a maladaptive, risky strategy. Such a suboptimal pattern of choice has been observed in numerous drug dependent populations, including cocaine (Verdejo-Garcia et al., 2007; Stevens et al., 2013), methamphetamine (Gonzalez et al., 2007; Wang et al., 2013), heroin (Verdejo-Garcia et al., 2007), marijuana (Bolla et al., 2005), alcohol (Bechara et al., 2001; Goudriaan et al., 2007), and polysubstance abusers (Grant et al., 2000), as well as pathological

gamblers (Goudriaan et al., 2005). Furthermore, poor choice behavior persists during drug withdrawal, and predicts treatment failure and addiction severity (Bechara et al., 2001; Wang et al., 2012; Stevens et al., 2013), implying that this cognitive deficit critically contributes to the maintenance of the addicted state.

Although multiple factors likely contribute to the manifestation of addiction, recognizing a prominent role for disordered decision making may open up new treatment approaches (Bechara, 2003; Bechara, 2005). However, the nature of the relationship between poor decision making and SUD is difficult to determine from clinical data, in which myriad environmental and circumstantial factors may predominate. Animal models can play a vital role in this regard (Potenza, 2009). We previously developed a rat gambling task (rGT), based on the IGT, performance of which depends on similar neural systems across species (Zeeb et al., 2009; Zeeb and Winstanley, 2011; Paine et al., 2013; Zeeb and Winstanley, 2013). Just as in the IGT, favoring the tempting “high risk- high reward” options results in significantly less reward over time; the advantageous strategy is to choose options associated with smaller per trial gain, but also lower punishments. Although most rats choose optimally, some instead exhibit a disadvantageous preference for the risky options. Here, we test the hypothesis that such risky choice represents a cognitive endophenotype for addiction vulnerability using the cocaine self-administration model of substance use, and the incubation of craving assessment of cue-driven drug seeking. Furthermore, concomitant rGT sessions also allowed us to determine whether the process of cocaine intake or withdrawal differentially affected decision making in risk-preferring versus optimal decision-makers.

3.2 Additional Methods

Subjects

Subjects were male Long-Evans rats (Charles River Laboratories, St. Constant, Canada). As risk-preferring rats make up only ~22% of the population, a total of 4 separate cohorts of 16 rats (64 in total) were trained on the rGT in succession, of which 28 were used in the current experiment (14 risk-preferring, 14 optimal choosers; see statistical analyses). Optimal rats were included until a sufficient number of rats for behavioural analysis was obtained. The remaining 36 optimal rats were reassigned to other ongoing behavioural experiments. Animals weighed between 275-300 g upon arrival, and were maintained at approximately 85% of their free-feeding weight by restricting their food to 14 g of rat chow per day. Water was available *ad libitum*.

Incubation of craving

24hrs following the last self-administration session, rats were placed into the self-administration chamber for 1 hr to measure responding to cocaine-paired cues. Pressing the active lever resulted in presentation of the light-tone cues previously associated with cocaine administration, followed by a 1 s inter-trial-interval, but no drug was administered at any time. This test was repeated after 30 days (Grimm et al., 2001). rGT sessions continued in the morning during this withdrawal period, but the animals remained in their home cages at all other times.

Statistical Analyses

All statistical analyses were completed using SYStat 12.0 software (Chicago, IL, USA) as previously described (see general methods). Animals with a mean positive score at baseline were designated as “optimal” (Figure 3.1; cocaine n=6, saline n=8), whereas rats with negative scores were classified as “risk-preferring” (cocaine n=6, saline n=8). This between-subjects factor (risk-preference) was included in all analyses.

The total responses on each lever from cocaine self-administration and cue-induced drug seeking test sessions were subject to repeated-measures ANOVA with risk-preference and drug as between-subjects factors, and session and lever as within-subjects variables. The magnitude of incubation of craving (#active lever presses on day 30 - #active lever presses on day 1 of withdrawal) was also analysed, and any correlation with the change in decision making caused by cocaine self-administration (baseline score – post self-administration score) was determined in cocaine-exposed animals.

3.3 Results

Baseline behavior

In keeping with previous studies (Zeeb et al., 2009), optimal and risk-preferring animals demonstrated distinct but stable choice preferences (data not shown; choice: $F_{2,64} = 12.74$, $p < 0.001$; choice x risk-preference: $F_{2,64} = 12.18$, $p < 0.001$; session: $F_{2,52} = 0.91$, $p = 0.31$) with optimal rats choosing P2 significantly more than risk-preferring animals ($t(26) = 5.65$, $p < 0.001$) and risk-preferring animals selecting P3 and P4 (P3: $t(26) = -2.43$, $p = 0.02$; P4: $t(26) = -3.44$, $p = 0.002$). P1 did not significantly differ between the two main risk-preferences (P1: $t(26) = -$

1.09, $p = 0.29$). All measures were statistically stable over the final 3 baseline sessions prior to self-administration (all $F_{2,52} < 2.34$, $p > 0.11$).

The score variable was significantly lower in the risk-preferring rats, indicative of an elevated preference for the risky, disadvantageous options. (Table 3.1; risk-preference: $F_{1,26} = 45.96$, $p < 0.001$; session: $F_{2,52} = 0.09$, $p = 0.90$; session x risk-preference: $F_{2,52} = 0.55$, $p = 0.58$). In concordance with previous data, risk-preferring animals also made more premature responses (Table 3.1; $F_{1,26} = 15.90$, $p < 0.001$). While risk-preferring rats completed fewer trials ($F_{1,26} = 8.45$, $p = 0.001$), this likely reflects the longer penalty periods incurred by these animals as a result of poor decision making and higher levels of impulsive action, rather than reduced motivation for reward, as these animals also made fewer omissions ($F_{1,26} = 11.84$, $p = 0.002$), and were faster to choose between the options (risk-preference: $F_{1,26} = 10.55$, $p = 0.003$) consistent with previous results (Barrus et al., 2015a). As all rats collected larger rewards more quickly, this resulted in an artificially lower average reward collection latency in risk-preferring rats that was simply an artefact of their choice bias; these rats were not actually any faster than optimal decision-makers to collect the larger or smaller rewards (Table 3.1; risk-preference: $F_{1,26} = 15.70$, $p = 0.0007$; choice- $F_{3,27} = 5.28$, $p = 0.005$; choice x risk-preference- $F_{3,27} = 0.33$, $p = 0.80$).

Self-administration

Responding on the active lever increased over the 10 self-administration sessions in animals responding for cocaine, but not saline (Figure 3.2A; lever: $F_{1,24} = 27.19$, $p < 0.001$; drug x lever x session: $F_{5,119} = 3.52$, $p = 0.005$). While this steady increase in response rates was similar across the cohort (session x risk-preference: $F_{6,58} = 0.27$, $p = 0.95$), risk-preferring animals pressed significantly more on the cocaine-paired lever overall (risk-preference: $F_{1,10} =$

6.24, $p = 0.03$). However, there was no significant difference in the number of cocaine infusions received across subgroups (Figure 3.2B; risk-preference: $F_{1,10} = 3.21$, $p = 0.10$; session x risk-preference: $F_{6,58} = 0.39$, $p = 0.87$). In animals self-administering saline, active lever pressing decreased over sessions in all rats (risk-preference $F_{1,14} = 1.79$, $p = 0.20$; session: $F_{5,68} = 5.61$, $p < 0.001$; session x risk-preference: $F_{5,68} = 1.07$, $p = 0.39$). Inactive lever pressing did not differ across risk-preference groups in either drug condition (Figure 3.3; risk-preference: $F_{1,24} = 0.01$, $p = 0.92$, drug x risk-preference: $F_{1,24} = 1.32$, $p = 0.26$).

rGT Performance

Risk-preferring rats' performance of the rGT became increasingly more maladaptive during the concurrent self-administration phase of the experiment, as indicated by a further decrease in the score (Figure 3.4B; session x drug x risk-preference: $F_{5,113} = 2.73$, $p = 0.02$; cocaine only- session x risk-preference: $F_{5,47} = 3.71$, $p = 0.007$; session- risk-preferring: $F_{6,31} = 3.24$, $p = 0.01$). In contrast, choice preference did not change in the optimal group even though these rats were ingesting comparable amounts of cocaine (optimal, session: $F_{4,20} = 1.64$, $p = 0.20$). Self-administration of saline did not alter performance of the rGT in either group (Figure 3.4a; $F_{4,20} < 1.64$, $p > 0.20$).

Although premature responding fluctuated during this phase of the experiment across the entire cohort, this variability was not isolated to any one risk-preference group or drug condition (Figure 3.5; Table 3.2; session: $F_{7,159} = 2.66$, $p = 0.01$; session x risk-preference: $F_{7,159} = 1.58$, $p = 0.15$; session x drug: $F_{7,159} = 0.41$, $p = 0.88$), and analyses of each risk-preferences' performance revealed no differences across sessions (all $F < 1.76$, $p > 0.18$). This statistical anomaly therefore likely reflects a general increase in behavioural variation in this measure, perhaps

caused by alterations in the rats' daily routine and environment. However, all other aspects of performance remained stable during this epoch (Table 3.2; session x risk-preference x drug: $F_{8,192} < 1.80$, $p > 0.18$).

The decline in optimal decision making was maintained during withdrawal, with neither an improvement nor further impairment observed in risk-preferring rats, or any other group (Figure 3.6A-B; session: $F_{2,46} = 1.89$, $p = 0.16$; session x risk-preference: $F_{2,46} = 0.32$, $p = 0.72$; session x drug: $F_{2,46} = 0.12$, $p = 0.89$). Somewhat in keeping with previous reports that motor impulsivity is exacerbated during withdrawal, risk-preferring rats that had self-administered cocaine made significantly more premature responses towards the midpoint of withdrawal (Table 3.3; session x risk-preference x drug: $F_{2,48} = 7.03$, $p = 0.002$; session- risk-preferring: $F_{2,10} = 5.14$, $p = 0.03$, all other groups: all $F_s < 2.66$, $p > 0.10$), although this had normalised by the end of the 30 day period (24 hr vs 30 days withdrawal: session x risk-preference x drug: $F_{1,24} = 1.20$, $p = 0.28$). The number of omissions also tended to vary during this experimental epoch (table 3.3; $F_{1,35} = 2.98$, $p = 0.07$), but subsequent within drug group analyses revealed no significant changes in responding (session x risk-preference: $F_{1,14} < 3.13$, $p > 0.09$). Similarly, choice and collection latencies also demonstrated significant changes during the withdrawal period (choice latency- session x drug x risk-preference: $F_{2,46} = 3.52$, $p = 0.04$; collection latency- $F_{2,46} = 5.07$, $p = 0.01$), but there were no significant differences within each drug group (session x risk-preference: $F < 2.38$, $p > 0.11$). As seen in self-administration, differences between risk-preference groups were also maintained throughout withdrawal for omissions, trials, and response latencies ($F > 4.12$, $p < 0.02$). The only other notable behavioural change observed during withdrawal was an increase in trials completed by optimal rats that had previously self-

administered cocaine (session x risk-preference x drug: $F_{2,46} = 3.69$, $p = 0.03$; session- optimal $F_{2,10} = 4.43$, $p = 0.04$; all other groups: all $F_s < 1.20$, $p > 0.34$).

Incubation of Craving

As expected, active lever responding increased after 30 days of withdrawal from cocaine across risk-preference groups (Fig. 3.7A; session x risk-preference x drug: $F_{1,24} = 5.15$, $p = 0.03$). Only a weak correlation was observed between the total number of active lever responses on day 30 and the total number of active lever presses during self-administration, suggesting that this incubation of craving effect reflects a process at least partially distinct from the baseline tendency to respond on the active lever during self-administration sessions (Figure 3.8; $r = 0.51$, $p = 0.09$). Analysis of responding between the cocaine optimal and risk-preferring animals revealed that incubation of craving was more pronounced in the risk-preferring group (session: $F_{1,10} = 18.90$, $p = 0.001$; session x risk-preference: $F_{1,10} = 5.86$, $p = 0.04$). Although the raw number of active lever presses was not significantly different between risk-preferring and optimal decision makers at the 30 day time point, we took advantage of the higher power made possible through this within-subjects design to determine the degree to which each individual rats' responding increased from the beginning to the end of withdrawal. This revealed a markedly elevated incubation of craving in risk-preferring rats (Fig. 3.7C; risk-preference: $F_{1,24} = 8.22$, $p = 0.008$; drug: $F_{1,24} = 11.97$, $p = 0.002$; risk-preference x drug: $F_{1,24} = 5.15$, $p = 0.03$; cocaine- risk-preference: $F_{1,10} = 5.86$, $p = 0.04$), suggesting enhanced susceptibility to the ability of drug-paired cues to promote relapse in these animals. Furthermore, the magnitude of this incubation of craving correlated with the increase in risky decision making observed during cocaine self-administration (Fig. 3.7D; $r = 0.61$, $p = 0.03$). Saline-experienced animals also

demonstrated a much smaller but statistically significant increase in active lever presses, although this did not differ between optimal and risk-preferring animals (session: $F_{1,14} = 10.13$, $p = 0.007$; session x risk-preference: $F_{1,14} = 0.68$, $p = 0.42$).

Inactive lever pressing also tended to increase across the two time points (Fig. 3.7B; session x risk-preference x drug: $F_{1,24} = 4.34$, $p = 0.05$), an effect that is largely attributed to a trend-level increase in lever-pressing in the cocaine-experienced risk-preferring rats (cocaine only- risk-preference: $F_{1,10} = 6.79$, $p = 0.03$; session x risk-preference: $F_{1,10} = 4.30$, $p = 0.07$; risk-preferring- session: $F_{1,5} = 4.75$, $p = 0.08$; optimal $F_{1,10} = 1.96$, $p = 0.19$). However, responding was significantly lower on the inactive than active lever, and may represent a general invigoration of behavior caused by exposure to the drug cue in the risk-preferring animals, or even frustration in response to the lack of concomitant drug delivery. No change in responding on the inactive lever was seen in saline controls (session: $F_{1,14} = 5.77$, risk-preference: $F_{1,14} = 2.58$, $p = 0.13$; $p = 0.03$; session x risk-preference: $F_{1,14} = 0.001$, $p = 0.98$).

3.4 Discussion

Here we show unequivocally, for the first time, that subjects making risky, maladaptive decisions at baseline are differentially and adversely affected by cocaine self-administration in a manner concordant with a pro-addictive phenotype. Animals identified as risk-preferring on the rGT made more responses on the drug-paired lever as compared to optimal decision-makers, and their decision making became more biased towards the maladaptive, risky options during the diurnal periods in which cocaine was self-administered. In contrast, the choice pattern of optimal rats remained consistent and advantageous, despite ingesting comparable amounts of cocaine. Risk-preferring rats also exhibited greater incubation of craving, and the degree to

which cocaine self-administration enhanced risky choice significantly correlated with this measure of cue-induced drug seeking. Hence, risky decision making may reflect a particularly important marker of addiction vulnerability.

As expected (Barrus et al., 2015a), risk-preferring rats were also quicker to make decisions and made more premature responses at baseline. High levels of such motor impulsivity in rats is also associated with a behavioural pattern representative of addiction as opposed to simple drug-taking (Belin et al., 2008; Economidou et al., 2009). In the current study, premature responding did spike mid-way through the withdrawal from cocaine in risk-preferring rats, somewhat similar to a previous report (Winstanley et al., 2006). However, unlike the observed elevations in risky choice, this form of impulsivity was not exacerbated either during cocaine self-administration, or throughout withdrawal. Our results, although in general concordance with the view that high motor impulsivity reflects aspects of addiction vulnerability, therefore suggest that the exacerbation of poor decision making in risk-preferring individuals uniquely reflects behavioural changes central to the addicted state, above and beyond the role played by behavioural disinhibition

Given that the rGT and self-administration sessions were run in completely distinct chambers using different manipulanda, it is unlikely that contextual conditioning caused by cocaine delivery could have contributed to the impairments in decision making, particularly as these deficits were observed selectively in risk-preferring rats. High levels of impulsive choice as measured by greater-than-average preference for smaller-sooner than larger-later reward on delay-discounting tasks, has also been identified as both a cause and consequence of cocaine self-administration (Perry et al., 2005; Mendez et al., 2010; Mitchell et al., 2014a). However, unlike in the current study, highly impulsive rats are not uniquely affected by cocaine- this form

of impulsivity either increases across the cohorts tested, regardless of baseline choice patterns, or is not further exacerbated in highly impulsive rats. A similar pattern is observed in a probabilistic reward/punishment paradigm, with risky choice increasing universally in all rats after cocaine self-administration (Mitchell et al., 2014b). Furthermore, recent studies have dissociated changes in impulsive choice from cue-induced relapse, both at a phenomenological and pharmacological level; drugs that can reduce or enhance cue or context-induced reinstatement do not concurrently impact impulsive decision making, and vice-versa (Broos et al., 2012; Broos et al., 2015). Risky decision making, as assessed by the rGT, is again somewhat unique in that adverse consequences of cocaine intake are selectively observed in risk-preferring rats, and further impairments in choice are concordant with the manifestation of relapse vulnerability as measured by incubation of craving. Such a conclusion is consistent with observations that substance abusers' risky decision making on the IGT was maintained through withdrawal (Wang et al., 2013) and was a stronger predictor of relapse than impulsive choice (De Wilde et al., 2013). In terms of building the validity of this putative cognitive marker of addiction vulnerability, it will be important to evaluate the impact of long access cocaine self-administration sessions, in which drug intake escalates and "bingeing" can occur, thereby more closely modeling the pattern of drug intake seen in SUD.

One factor that may have contributed to the comparatively robust nature of our findings is that, unlike many other behavioural studies in which baseline differences are exploited (e.g. (Perry et al., 2008; Robinson et al., 2009; Besson et al., 2010; Mitchell et al., 2014b), we did not have to resort to arbitrary markers such as a median split or inter-quartile analysis to assign rats as either risk-preferring or optimal decision makers; defining risk preference as a negative score on the rGT is both objective and completely independent of the average behavioural output of a

current or historical cohort. While this is a desirable feature of the current methodology, it inevitably requires the screening of large numbers of rats to obtain a sufficient sample size of risk-preferring animals. However, the proportion of individuals showing this behavioural pattern (22%) is comparable to the prevalence of addiction seen in humans, estimated as ~18%, further increasing the face validity of this model.

It is also worth emphasising that, as any rat with a negative score was designated risk-preferring, there was some variation in the degree of risk preference observed. It is also clear that the magnitude of the change in choice behavior that resulted from cocaine self-administration varied, and this variation tracked that observed in the assessment of incubation of craving. Why some rats are more affected than others is currently unknown, but may indicate the presence of factors capable of promoting either resilience or vulnerability to the deleterious effects of cocaine self-administration. Such a possibility should be considered in future work aiming to elucidate the neurobiological basis underlying the behavioural impact of cocaine in risk-preferring rats. More specifically, such studies should be designed with sufficient power to detect variation in biochemical markers *within* this risk-preferring cohort, as well as between risk-preferring and optimally-choosing animals, that can then be mapped to the individual differences in the behavioural phenotype.

Risk-preferring rats responded more times on the active lever than optimal rats, yet received statistically indistinguishable, albeit visibly higher, numbers of infusions. Although this null effect could simply reflect a lack of power, it may also offer insight into the mechanism underlying the higher drug-responsivity observed in risk-preferring rats. Theoretically, this behavioural effect represents a qualitatively similar response to that observed in the optimal group, yet of a larger magnitude, potentially indicative of a sensitized response to cocaine. As to

the origins of such sensitization, recent data indicate that repeated exposure to conditioned stimuli associated with probabilistic reward delivery, or responding for probabilistic as opposed to guaranteed rewards, can enhance locomotor sensitization to amphetamine (Singer et al., 2012; Zack et al., 2014). This raises the intriguing possibility that repeatedly engaging in risky decision making actually contributes to a sensitized response to psychostimulants (Zack and Poulos, 2009), rather than there being an innate difference between animals that go on to be risk-preferring versus optimal decision makers. Such a hypothesis remains open to empirical verification, but could have implications for why conditions hallmarked by persistent, elevated risky choice, such as gambling and bipolar disorders, are highly comorbid with substance use disorder.

Exacerbation of an already maladaptive choice strategy may reflect reduced cognitive flexibility or perseveration. Numerous studies have reported deficits in reversal learning and other indications of cognitive rigidity following chronic cocaine, largely attributed to a relatively insensitive and underactive prefrontal (PFC) network, mediated at least in part by deficient signaling of current cue-outcome associations in the basolateral amygdala (BLA) (Stalnaker et al., 2007a; Stalnaker et al., 2007b; Stalnaker et al., 2009; Lucantonio et al., 2012; Cervantes et al., 2013). Lesioning the BLA in optimal decision-makers increases choice of P3 and P4, whereas disconnection of the BLA from the orbitofrontal cortex (OFC) impairs task acquisition (Zeeb and Winstanley, 2011, 2013). BLA lesions also attenuate the impact of losses on subsequent decisions in a rodent loss-chasing task, positing a specific role of the BLA in influencing decision making through the representation of aversive consequences (Tremblay et al., 2014). Similarly, repeated administration of the psychostimulant amphetamine reduces the impact that conditioned aversive stimuli exert on instrumental behavior (Tse et al., 2011),

potentially due to impairments in the ability of BLA neurons to inhibit firing in the PFC via activation of local interneurons (Tse et al., 2011). One hypothesis, therefore, is that impairments in BLA-PFC/OFC signaling could be evident in risk-preferring rats, and exacerbated by cocaine self-administration, resulting in persistent selection of the risky options despite the ensuing negative consequences.

“Silent synapses”, in which NMDA receptor expression at the synaptic membrane is upregulated in the absence of robust increases in AMPA receptors, have been detected within projections between the ventromedial prefrontal (infralimbic) cortex and nucleus accumbens (NAC) core, as well as within the BLA-NAC shell pathway, following cocaine self-administration and critically contribute to the expression of incubation of craving (Lee et al., 2013; Ma et al., 2014). Given that the BLA and medial PFC influence risky choice on the rGT (Zeeb and Winstanley, 2011; Paine et al., 2013), such a signaling pathway may likewise contribute to the maintenance of cocaine-induced increases in risky decision making on the rGT, and its relationship to incubation of craving. However, future studies investigating these mechanisms are required to discern the involvement of these circuits.

Understanding the neurobiological basis mediating the relationship between maladaptive decision making and addiction may offer much-needed insight into the etiology and trajectory of SUD. Future studies can now capitalize on this demonstration of a novel and readily-quantifiable cognitive endophenotype for SUD. The fact that addicts must choose to continually engage in the addiction for use disorders to persist has led some to view addicts as deserving of retribution rather than treatment (Boyarsky et al., 2002). This demonstration of a robust interaction between poor choice on a gambling-like task and enhanced drug seeking in an animal model of cocaine addiction suggests that this relationship arises not simply from environmental

or circumstantial factors, but instead from physiological alterations in brain function within risk-preferring individuals that are uniquely and adversely affected by drug intake.

3.5 Tables

<i>Behavioural Measure</i>	<i>Optimal</i>	<i>Risk-preferring</i>
Score Variable	69.77 ± 9.81	-22.29 ± 8.59
Premature Responding	8.56 ± 1.44	28.11 ± 4.26
Omitted Responses	2.64 ± 0.64	0.72 ± 0.46
Trials Completed	98.76 ± 8.50	64.40 ± 4.51
Choice Latency Average	1.59 ± 0.16	0.82 ± 0.20
Collection Latency Average	1.04 ± 0.10	0.52 ± 0.06

Table 3-1 Behavioral performance on the rGT at baseline.

All values are group averages for final three baseline sessions ± standard error of the mean (SEM)

<i>Behavioural Measure</i>	<i>Saline Optimal</i>	<i>Saline Risk-Preferring</i>	<i>Cocaine Optimal</i>	<i>Cocaine Risk-Preferring</i>
Premature Responding	11.99 ± 3.09	22.22 ± 5.07	4.54 ± 1.04	18.09 ± 4.08
Omitted Responses	3.09 ± 0.91	1.01 ± 0.84	4.67 ± 1.39	1.39 ± 0.52
Trials Completed	102.45 ± 6.62	71.76 ± 6.08	100.80 ± 9.91	61.65 ± 7.55
Choice Latency	1.43 ± 0.19	0.99 ± 0.31	2.21 ± 0.28	1.12 ± 0.33
Collect Latency	0.96 ± 0.09	0.55 ± 0.07	1.11 ± 0.13	0.83 ± 0.21

Table 3-2 rGT performance over the 10 self-administration sessions.

Values presented are averages over 10 self-administration sessions ± SEM.

<i>Behavioural Measure</i>	<i>Withdrawal Timepoint</i>	<i>Saline Optimal</i>	<i>Saline Risk-Preferring</i>	<i>Cocaine Optimal</i>	<i>Cocaine Risk-Preferring</i>
Premature Responding	1 Day	10.93 ± 3.78	17.89 ± 7.84	3.42 ± 0.62	9.21 ± 3.66*
	15 Days	18.01 ± 4.93	11.96 ± 5.36	4.22 ± 2.23	19.85 ± 6.52*
	30 Days	15.48 ± 3.34	13.24 ± 6.42	5.98 ± 2.56	11.66 ± 2.74*
Omitted Responses	1 Day	4.00 ± 2.20	0.75 ± 0.49	9.16 ± 3.78	1.33 ± 0.84
	15 Days	3.75 ± 2.20	0.87 ± 0.51	5.50 ± 1.33	0.50 ± 0.50
	30 Days	0.86 ± 0.70	0.37 ± 0.70	1.67 ± 0.61	1.67 ± 0.95
Trials Completed	1 Day	112.38 ± 11.23	74.67 ± 7.60	104.83 ± 13.50*	60.02 ± 9.55
	15 Days	111.39 ± 10.00	83.02 ± 8.66	126.83 ± 10.54*	56.37 ± 5.95
	30 Days	112.16 ± 8.43	73.92 ± 9.56	128.83 ± 12.55*	61.85 ± 8.00
Choice Latency	1 Day	1.38 ± 0.25	0.97 ± 0.23	1.93 ± 0.31	0.92 ± 0.26
	15 Days	1.09 ± 0.27	1.14 ± 0.29	1.99 ± 0.27	0.72 ± 0.23
	30 Days	0.93 ± 0.17	1.70 ± 0.29	1.70 ± 0.29	0.78 ± 0.15
Collect Latency	1 Day	0.97 ± 0.13	0.53 ± 0.08	1.12 ± 0.17	0.61 ± 0.10
	15 Days	0.87 ± 0.13	0.62 ± 0.08	1.19 ± 0.14	0.51 ± 0.12
	30 Days	0.86 ± 0.09	0.50 ± 0.05	1.09 ± 0.09	0.54 ± 0.08

Table 3-3 rGT performance from the beginning, middle, and end of the 30-day withdrawal period.

Values are averages per withdrawal timepoint ± SEM. Bold values denoted with an asterisk have a p-value < 0.05.

3.6 Figures

Figure 3-1 Distribution of rGT score of rats used in experiment (n=28).

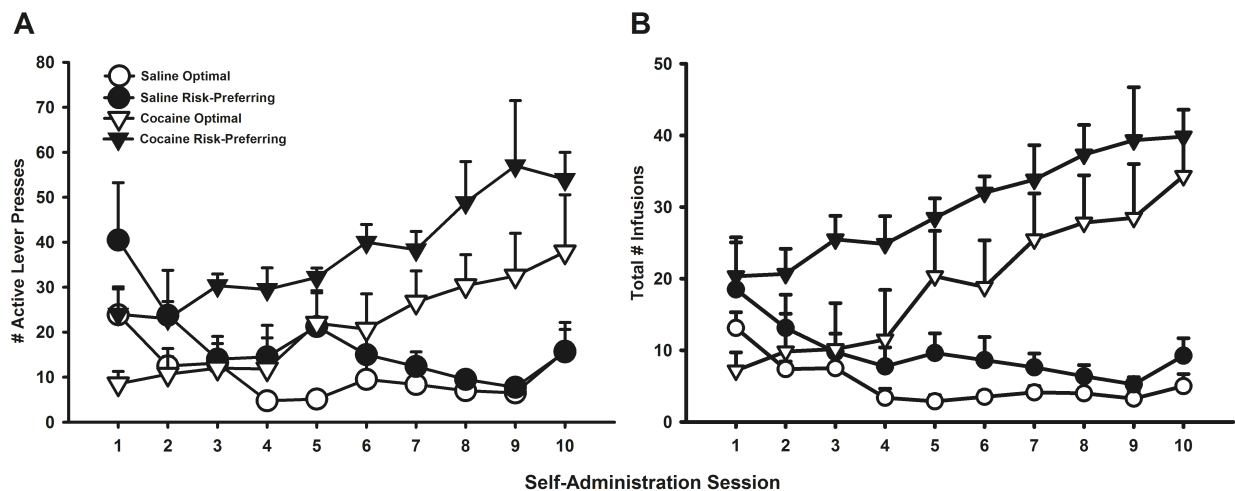
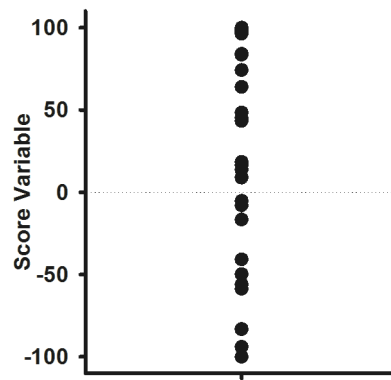


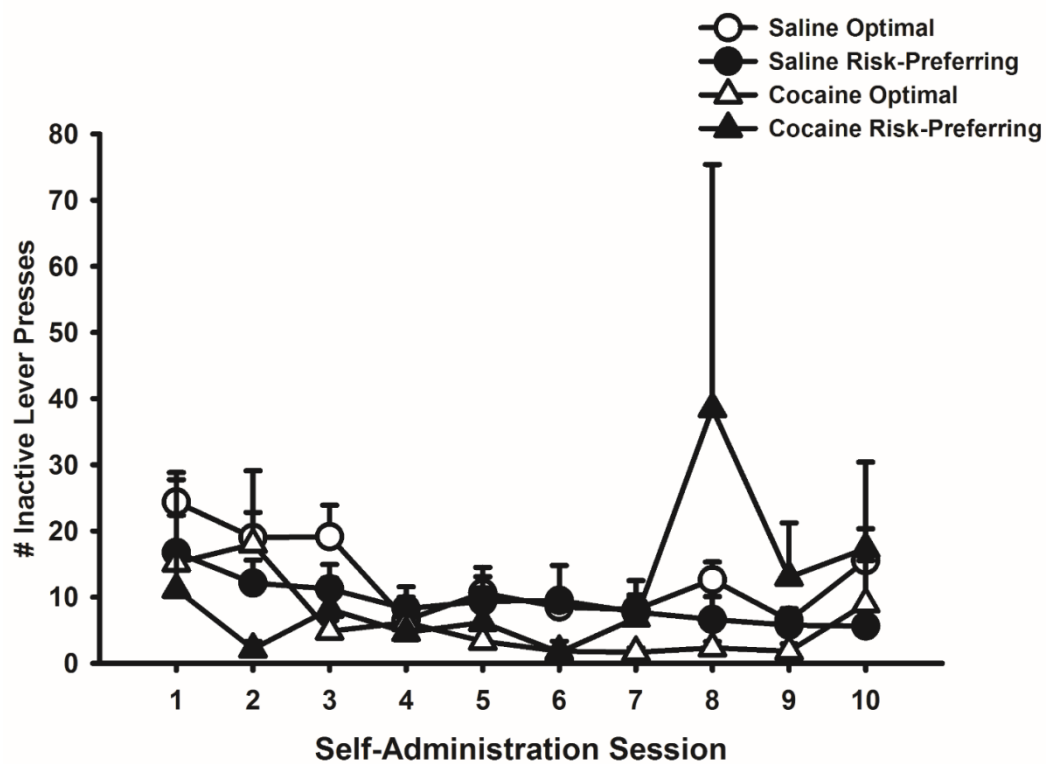
Figure 3-2 Responding during 3 hr drug self-administration sessions.

(A) Number of active lever presses across the subgroups over the self-administration period.

Both risk-preference groups self-administering cocaine developed a preference for the active lever over the 10 self-administration sessions, but risk-preferring rats pressed the active lever significantly more than optimal rats across all sessions. Animals self-administering saline decreased responding on the lever over the 10 sessions, with no significant difference observed

between choice-preference groups. (B) Number of infusions received during self-administration sessions. As expected, cocaine animals received significantly more infusions than saline animals. However, there was no significant difference between cocaine optimal and risk-preferring animals' cocaine intake. Therefore, the changes in decision making observed in risk-preferring rats cannot be attributed to elevated consumption. Data are presented as mean \pm SEM.

Figure 3-3 Number of inactive lever presses during self-administration sessions.



Responding on the inactive lever did not differ across groups in either drug condition. Data are presented as mean \pm SEM.

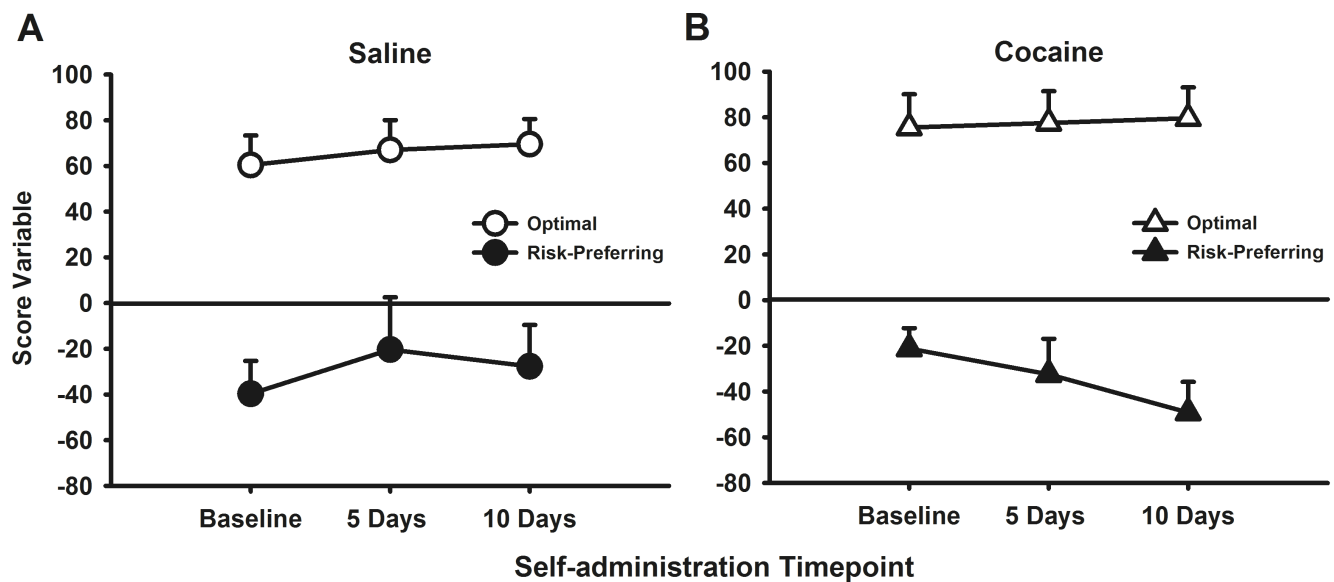


Figure 3-4 Decision making as measured by the score variable during the self-administration period.

(A) As expected, saline animals' decision making remained unchanged. (B) However, while the advantageous choice pattern of optimal decision-makers self-administering cocaine did not alter, risk-preferring rats made significantly more risky and maladaptive choices during the self-administration epoch. Data are presented as mean per self-administration timepoint \pm SEM.

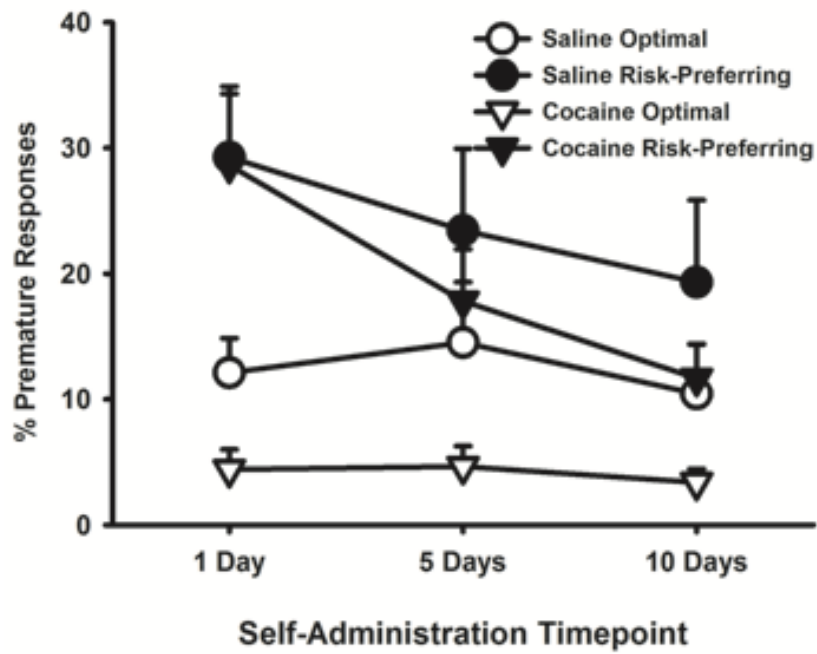


Figure 3-5 Premature responses during self-administration.

Impulsivity varied during this epoch but did not significantly change from baseline. Values presented are averages per self-administration timepoint \pm SEM.

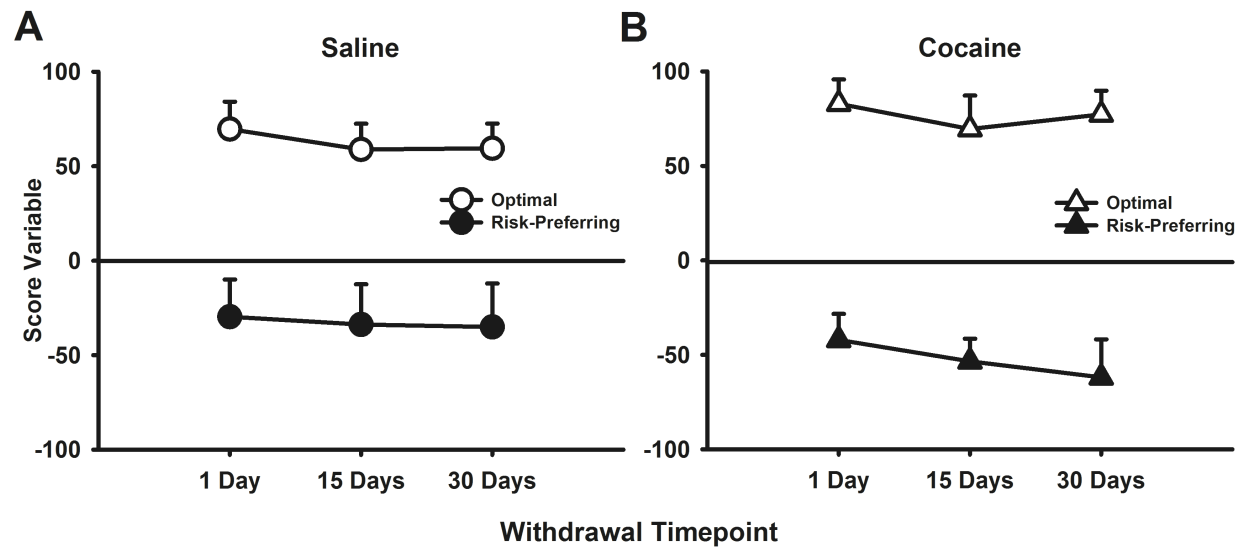


Figure 3-6 Decision making as measured by the score variable during the withdrawal period.

(A) As expected, saline animals' decision making remained unchanged. (B) In keeping with the human literature, cocaine risk-preferring animals' score remained stable during this time, and did not recover to pre-drug exposure levels. Data are presented as means per withdrawal timepoint \pm SEM.

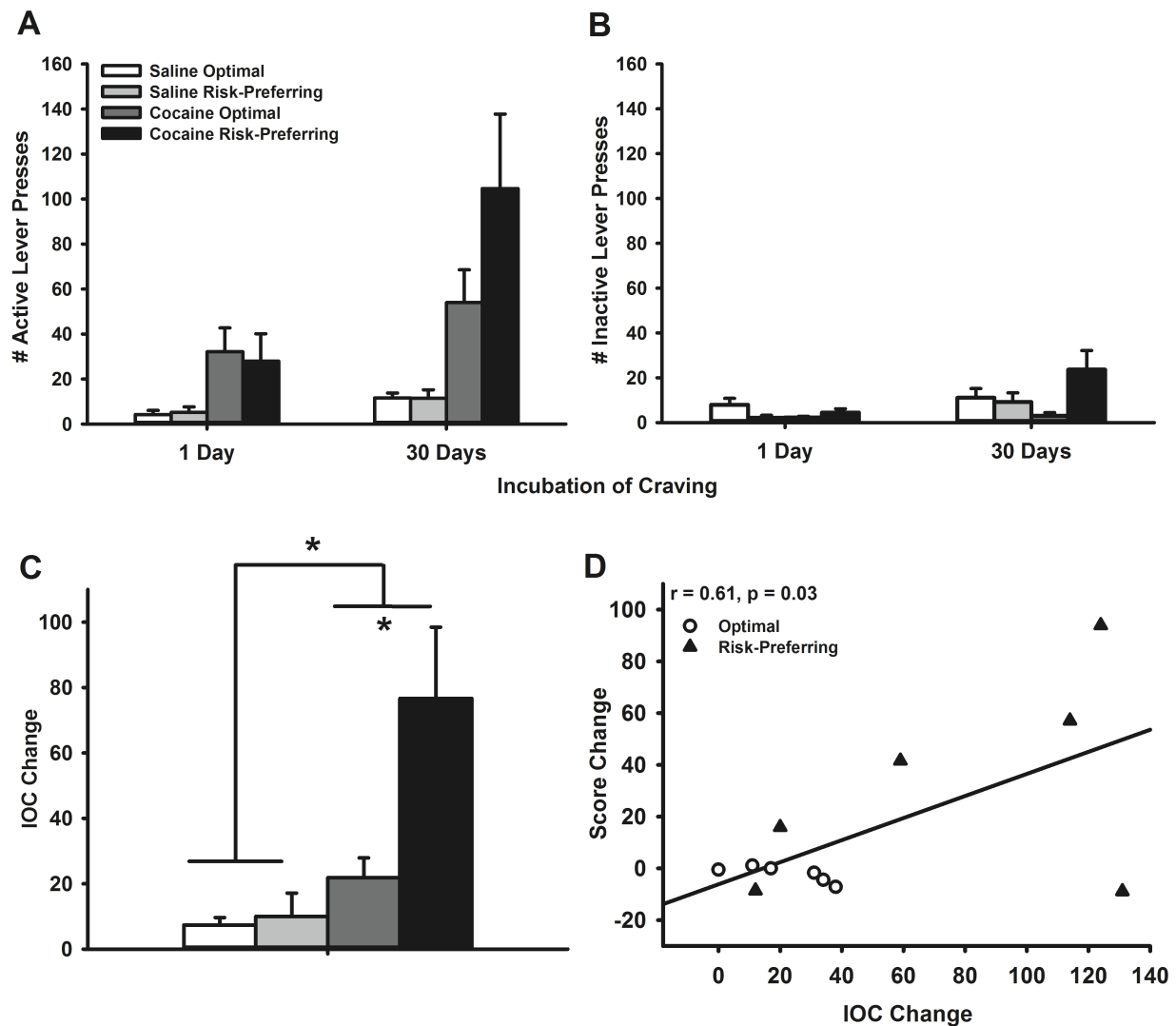


Figure 3-7 Number of responses on active and inactive levers during the incubation of craving test sessions, comparing cue-induced drug seeking behaviour at the start and end of the 30 day withdrawal period.

(A) Compared to saline animals, cocaine-exposed rats pressed the active lever significantly at both time points. Within the cocaine group, although optimal decision-makers increased responding on the active lever after 30 days of withdrawal, this incubation of craving effects was significantly greater in risk-preferring rats, indicative of greater relapse vulnerability in these

animals. (B) While inactive lever responding was unchanged in the saline risk-preference groups and cocaine optimal animals, cocaine risk-preferring rats did increase responding on the inactive lever during the incubation of craving paradigm, potentially the result of general invigoration of behavior caused by exposure to the drug cue. However, inactive lever presses were much lower than responses on the previously drug-paired lever. Data are presented as mean \pm standard error of the mean. (C) While absolute scores did not demonstrate significant subgroup differences between cocaine animals, the difference score (responses on day 30-responses on day 1 of withdrawal) reflected a significant increase in responding for cocaine-associated cues in cocaine risk-preferring rats. Data are presented as mean \pm standard error of the mean. (D) The degree of incubation of craving (IOC) observed was significantly correlated with the magnitude of the decrease in score, indicating a relationship between the cocaine-induced deterioration in decision making and relapse vulnerability. Data presented are those of individual rats within the cocaine optimal and risk-preferring subgroups. * denotes $p < 0.05$.

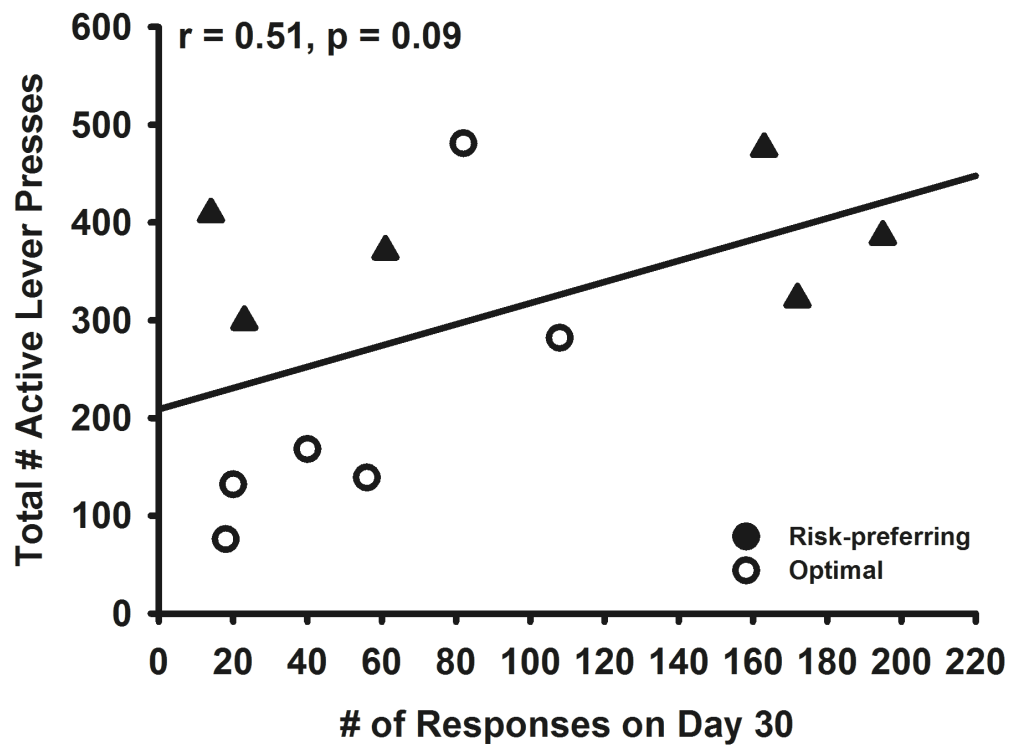


Figure 3-8 The correlation between total number of active lever presses and IOC response in cocaine animals.

Chapter 4: Experience with win-paired audiovisual cues confers greater responsivity for cocaine and drug-induced deficits in decision making.

4.1 Introduction

Addiction is a psychiatric illness characterized by compulsive drug seeking and relapse. Those who struggle with addiction exhibit poor cost/benefit decision making, which may play a crucial role in mediating the onset and maintenance of addiction (Bechara, 2003; Stevens et al., 2013; Wang et al., 2013). Indeed, heroin, cocaine, alcohol, and marijuana abuse are associated with impaired performance on the Iowa Gambling Task (IGT; (Rogers et al., 1999; Bolla et al., 2005; Verdejo-Garcia et al., 2007; Verdejo-Garcia and Bechara, 2009), a clinical assay of decision making. We have previously shown using the rat gambling task (rGT), a rodent analogue of the IGT, that animals who preferred the “risky” options of the task were uniquely and adversely affected by cocaine self-administration, as these animals showed heightened responding for drug, incubation of craving, and worsened decision making (Ferland and Winstanley, 2016). Investigating factors that promote risk-preference may provide significant insight into the etiology of addiction.

Drug-paired cues are thought to play a significant role in the maintenance of SUD as they can become imbued with “incentive salience,” whereby the cues themselves begin to elicit a response independent of reward delivery (Robinson and Berridge, 1993). The propensity to approach conditioned cues, or “sign-tracking”, has been previously associated with addiction susceptibility (Flagel et al., 2009; Flagel et al., 2010). Animals bred for heightened locomotor

response to cocaine exhibited greater sign-tracking behaviour for food and drug-paired cues, were more impulsive, and had low striatal dopamine D₂ receptor expression but high D₂ binding within the dorsal striatum, all hallmarks of addiction risk (Economidou et al., 2009; Volkow et al., 2009; Flagel et al., 2010; Molander et al., 2011; Belin et al., 2016; Worhunsky et al., 2017).

Multiple studies indicate cues may also influence cognitive bias and behaviour. For example, conditioned cues have been found to instigate relapse and persistent gambling (Epstein et al., 2006; Wang et al., 2012; Robinson et al., 2014; Limbrick-Oldfield et al., 2017), two situations where the decision to continue the behaviour is detrimental to the livelihood of the individual. Patients with Parkinson's disease who exhibit compulsive behaviours (i.e. gambling, shopping) while being treated with dopamine agonists rapidly learn associations between cues and probabilistic gains, and show greater ventral striatum activity, suggesting individuals with dysregulated dopamine systems may be particularly prone to cue-mediated learning (Voon et al., 2010). Our lab has previously shown that the addition of win-paired cues to the rGT that scale in complexity with the size of the reward earned (the "cued rGT"; crGT) augments choice of the disadvantageous options of the task (Barrus and Winstanley, 2016). When considered with extant literature on cue-mediated behaviours, these data indicate that cues can incentivize uncertain outcomes, as well as amplify maladaptive decision making in individuals who already have a predisposition for habitual and risky behaviour. Investigating whether cue-enhanced decision making may cultivate greater responding for drugs of abuse therefore merits exploration.

Perhaps the most challenging aspect of addiction treatment is maintaining abstinence. Approximately 40-60% of those who receive treatment for drug or alcohol abuse relapse within one year of exiting a treatment program (McLellan et al., 2000). Remarkably, poor IGT performance is predictive of treatment drop-out risk (Stevens et al., 2013). Reward-paired stimuli

have also been implicated in relapse. In methamphetamine abusers, disadvantageous decision making is exacerbated by drug-paired cues, and is associated with greater drug craving during abstinence, particularly within the first year of sobriety (Wang et al., 2012; Wang et al., 2013). Furthermore, those who have greater activation of mesolimbic reward structures after cue exposure express higher subjective cravings and are more likely to resume drug abuse (Wang et al., 2012; Li et al., 2015). Data from preclinical studies have shown single exposure to drug-paired cues and contexts is sufficient to reinstate drug seeking (Epstein et al., 2006), but is mitigated by individual differences in responsivity for cues or drug-primed reinstatement (Homberg et al., 2004). These data implicate decision making and cue reactivity in withdrawal, and suggest the two may work synergistically to promote relapse. Understanding how drug-induced cognitive deficits are maintained or change during withdrawal should be investigated to provide foundational understanding for the development of therapeutics for this critical stage of addiction.

In the current study, we sought to determine whether cue-induced risky decision making 1) promoted drug seeking, 2) was perturbed by cocaine self-administration, and 3) was affected during withdrawal within a subgroup of animals. Rats trained on the crGT underwent concurrent cocaine self-administration and decision-making sessions followed by 30 days of withdrawal. We predicted exposure to win-paired cues during the crGT would increase risky decision making, and rats that exhibited a preference for the risky options of the task would show enhanced drug seeking and increased disadvantageous choice after cocaine experience. Furthermore, these deficits would be maintained during withdrawal.

4.2 Additional Methods

Withdrawal period

Following completion of self-administration, a subgroup of animals (n=13) were put through a 30-day withdrawal period during which crGT performance was recorded 5-6 times per week. The study was concluded at the end of the withdrawal period.

Statistical Analyses

All statistical analyses were completed using SPSS 24.0 (IBM) as described previously (see general methods). Specific to this study, animals with a mean positive crGT score at baseline were designated as “optimal” (cocaine n=6, saline n=5), whereas rats with negative scores were classified as “risk-preferring” (cocaine n=8, saline n=7). This between-subjects factor (risk-preference) was included in all analyses. The total responses on each lever from cocaine self-administration were subject to repeated-measures ANOVA (two levels- active, inactive). Drug (n=14 cocaine, n=12 saline) was also included as a between-subjects variable for all self-administration and concurrent crGT behavioural analyses. For self-administration analyses, six rats were excluded due to loss of catheter patency. Sixteen animals had originally been planned for the withdrawal study, but due to loss of catheter (n=1) and death during surgery/ withdrawal (n=2), 3 animals were excluded from these analyses. Drug and risk-preference were included as between-subjects variables for withdrawal data (n=5 saline, n=8 cocaine; saline condition: optimal- n=2; risk-preferring- n=3; cocaine condition: optimal- n=3; risk-preferring- n=5).

4.3 Results

Self-administration

Compared to saline rats, cocaine animals exhibited a significant preference for the active lever over the 10-day self-administration period (Figure 4.1A; lever- $F_{1,20} = 30.034$, $p < 0.001$; lever x drug- $F_{1,20} = 11.531$, $p = 0.003$; session x drug- $F_{6,125} = 2.131$, $p = 0.052$; drug- $F_{1,20} = 9.872$, $p = 0.005$) as well as total number of infusions received (drug- $F_{1,22} = 40.303$, $p < 0.001$; session- $F_{7,144} = 1.209$, $p = 0.303$; session x drug- $F_{7,144} = 3.561$, $p = 0.002$). In contrast to previous work, risk-preferring and optimal rats had similar responding on the cocaine-paired lever throughout self-administration (interactions x risk-preference- $F < 1.360$, $p = 0.257$; cocaine only: risk-preference, lever x risk-preference, session x risk-preference, lever x session x risk-preference- $F < 1.734$, $p > 0.217$). Furthermore, while cocaine animals did exhibit a preference for the drug-paired lever (cocaine: lever- $F_{1,10} = 32.993$, $p < 0.001$), they did not gradually increase the total number of active lever responses (session- $F_{9,74} = 0.964$, $p = 0.466$) and only showed a trend increase in number of infusions received (Figure 4.1B; cocaine only: session- $F_{7,88} = 1.977$, $p = 0.064$; session x risk-preference- $F_{7,88} = 0.708$, $p = 0.672$; risk-preference- $F_{1,12} = 3.351$, $p = 0.092$) across self-administration sessions. This early drug-taking profile is atypical for self-administration studies (Belin-Rauscent et al., 2016), and suggests rapid acquisition. Saline rats exhibited significant decreases in both active lever pressing and infusions (Figure 4.1A-C, D-E; active lever: session- $F_{6,58} = 3.338$, $p = 0.007$; infusions: session- $F_{7,75} = 7.603$, $p < 0.001$), with saline risk-preferring rats showing a sharper decrease in infusions over the 10-day period (session x risk-preference- $F_{7,75} = 4.268$, $p < 0.001$). Inactive lever presses

were low for all rats and remained stable throughout the self-administration period (Figure 4.1 C, F; $F < 1.474$, $p > 0.232$).

rGT performance

Prior to self-administration, animals showed distinct decision-making preferences (Figure 4.4A-B; choice- $F_{3,66} = 7.000$, $p < 0.001$; choice x risk-preference- $F_{2,66} = 4.723$, $p = 0.005$ score: risk-preference- $F_{1,22} = 19.193$, $p < 0.001$). Specifically, optimal animals showed a preference for P2 and risk-preferring rats for P3 (independent samples t-test: P2- $t(24) = 2.673$, $p = 0.013$; P3- $t(24) = -2.444$, $p = 0.022$; P1 and P4- $t < 1.414$, $p > 0.17$). Optimal animals also completed significantly more trials, tended to make slightly fewer premature responses, and exhibited longer collection latencies compared to risky animals (table 4.1; trials: risk-preference- $F_{1,22} = 13.993$, $p = 0.001$; premature responses: risk-preference- $F_{1,22} = 4.015$, $p = 0.058$; collection latency: risk-preference- $F_{1,21} = 6.027$, $p = 0.023$). Although each individual cohort was fully counterbalanced for behaviour prior to self-administration, exclusion of animals due to catheter failure resulted in differences in the number of trials completed per session between saline and cocaine rats (table 4.1; drug- $F_{4,576}$, $p = 0.044$). Furthermore, optimal cocaine animals tended to show marginally higher levels of premature responding compared to saline counterparts (Table 4.1; saline only: risk-preference- $F_{1,10} = 4.359$, $p = 0.063$). No significant differences were observed across other task measures between drug or choice strategy groups (Table 4.1; all F 's < 2.696 , p 's > 0.115), and all variables were stable across sessions (all F 's < 2.792 , p 's > 0.073).

During rGT sessions run concomitantly with self-administration, all cocaine animals showed an increase in risky decision making as reflected by a decrease in score (Fig. 4.2 B-D; session- $F_{2,44} = 5.304$, $p = 0.009$; session x drug- $F_{2,44} = 2.782$, $p = 0.073$; drug- $F_{1,22} = 5.592$, $p =$

0.027; session x risk-preference, session x drug x risk-preference- $F < 0.834$, $p > 0.441$). This effect was most pronounced from Day 1 to Day 10 (session- $F_{1,22} = 6.750$, $p = 0.016$; session x drug- $F_{1,22} = 4.410$, $p = 0.047$). In contrast to findings with the uncued rGT (see Chapter 3), baseline risk-preference did not mediate drug-induced decision-making deficits, as both optimal and risk-preferring rats showed significant decreases in score during the self-administration period (Figure 4.2 B; cocaine: session- $F_{2,24} = 6.018$, $p = 0.008$; session x risk-preference- $F_{2,24} = 0.095$, $p = 0.909$). Importantly, although optimal decision-makers and risk-preferring animals received a similar number of infusions, there was no relationship between the amount of cocaine consumed during self-administration and worsening of score (see Fig. 4.3; cocaine only- $r = -0.046$, $p = 0.875$). In contrast, saline treated animals did not exhibit any significant changes in score (Figure 4.2 A; session, session x risk-preference- $F < 1.996$, $p > 0.162$; risk-preference- $F_{1,10} = 39.999$, $p < 0.001$).

In terms of altering choice of the specific options on the crGT, cocaine rats showed marked changes in decision making after self-administration (Figure 4.4; session x drug x risk-preference- $F_{2,44} = 3.664$, $p = 0.034$; session x choice x risk-preference- $F_{5,121} = 3.420$, $p = 0.005$). In cocaine rats, both optimal and risk-preferring animals exhibited alterations in choice preference (cocaine: session x choice- $F_{6,72} = 2.05$, $p = 0.07$), most prominently in risk-preferring animals (Figure 4.4 B; session x risk-preference; $F_{2,24} = 8.52$, $p = 0.002$) who showed a clear increase in preference for P3 and a decrease in choice of P2 during cocaine exposure (cocaine risk-preferring only- session x choice- $F_{4,30} = 3.132$, $p = 0.027$; paired samples t-test: Day 1 vs Day 6- P2, $t(7) = 2.708$, $p = 0.030$; P3, $t(7) = -2.293$, $p = 0.056$; Day 1 vs Day 10- P2, $t(7) = 3.972$, $p = 0.005$; P3, $t(7) = -3.138$, $p = 0.016$; all remaining tests- t 's < 1.337 , p 's > 0.223).

Optimal rats self-administering cocaine also showed some destabilization of choice preference (Figure 4.4 A; session- $F_{2,10} = 3.164$, $p = 0.086$; choice- $F_{3,15} = 7.712$, $p = 0.002$), and while visual inspection of the data shows a similar pattern of behaviour as risk-preferring animals, these shifts in choice were not statistically significant (session x choice- $F_{6,30} = 0.424$, $p = 0.857$), potentially due to highly variable preferences between individual rats. Saline optimal rats also showed some small but significant changes in choice during self- administration sessions (session x choice x risk-preference- $F_{3,31} = 3.465$, $p = 0.027$; saline optimal only: session x choice- $F_{5,24} = 2.835$, $p = 0.040$), although in favour of improved decision making by a significant increase in P2 and decreases in P1 and P3 on day 6 of self-administration (paired samples t-test: saline optimal- Day 1 vs Day 6, P1- $t(5) = 2.261$, $p = 0.073$; P2- $t(5) = -4.414$, $p = 0.007$; P3- $t(5) = 6.706$, $p = 0.001$; remaining tests- $t < 2.180$, $p > 0.082$). These changes were not present in saline risk-preferring animals (all F 's < 3.040 , p 's > 0.115).

Interestingly, cocaine self-administration differentially affected optimal and risk-preferring animals' impulsive responding (Fig 4.5 A-B; session x drug x risk-preference- $F_{2,44} = 3.442$, $p = 0.041$). While risk-preferring animals maintained baseline levels of responding (session, session x drug, drug- all F 's $< 2,171$, p 's > 0.136), optimal decision-makers self-administering cocaine showed a significant reduction in motor impulsivity compared to saline controls (session x risk-preference- $F_{2,20} = 6.963$, $p = 0.005$; saline rats- session, session x risk-preference- $F < 1.713$, $p > 0.206$; risk-preference- $F_{1,10} = 7.770$, $p = 0.019$; cocaine rats- session- $F_{2,20} = 4.430$, $p = 0.033$; session x risk-preference- $F_{2,20} = 7.345$, $p = 0.006$; risk-preference- $F_{1,12} = 0.005$, $p = 0.942$). In general, risk-preferring rats exhibited significantly faster collection latencies during self-administration (Table 4.2; risk-preference- $F_{1,22} = 15.189$, $p = 0.001$) and fewer trials completed (risk-preference- $F_{1,22} = 33.310$, $p < 0.001$), but were stable across self-

administration sessions ($F < 2.682$, $p > 0.081$). Omitted responses (session- $F_{2,44} = 2.937$, $p = 0.063$) and choice latencies (session- $F_{1,44} = 3.175$, $p = 0.054$) varied across self-administration sessions, but not specifically in one drug or risk-preference group ($F < 3.098$, $p > 0.092$), suggesting alterations in daily running schedules may have influenced responding on these measures.

Withdrawal

A subgroup of animals was put through a 30-day withdrawal period to determine whether decision making would be influenced by abstinence. Interestingly, decision making varied during this epoch, with differential responding by drug (Figure 4.6A-B; choice: session- $F_{2,18} = 3.612$, $p = 0.048$; choice- $F_{3,26} = 4.547$, $p = 0.011$; choice x risk-preference- $F_{3,26} = 4.183$, $p = 0.017$; session x choice- $F_{6,54} = 2.195$, $p = 0.058$; session x choice x drug = $F_{6,54} = 2.237$, $p = 0.053$). Subsequent analyses showed that saline animals' decision making was unaltered (Figure 4.6; $F < 2.40$, $p > 0.135$), whereas cocaine rats exhibited significant changes in choice, most likely attributable to cocaine optimal animals reacquiring an advantageous decision-making profile (Figure 4.6A; cocaine: session x choice- $F_{5,31} = 4.071$, $p = 0.005$; session x choice x risk-preference- $F_{5,31} = 2.269$, $p = 0.07$; choice x risk-preference – $F_{3,18} = 3.863$, $p = 0.027$; all remaining $F < 1.872$, $p > 0.196$). These changes in choice preference did not result in an overall significant change in score (Table 4.3; all $F < 2.065$, $p > 0.199$), likely due to high levels of variability within the subgroup. This improvement in decision making was accompanied by an increase in number of trials completed for cocaine optimal rats (session x risk-preference- $F_{2,18} = 5.504$, $p = 0.014$; session x drug- $F_{2,18} = 6.290$, $p = 0.008$; cocaine optimal: session $F_{2,4} = 7.620$, $p = 0.043$; saline and cocaine risk-preferring- all F 's < 1.406 , p 's > 0.416).

Choice latencies were also significantly reduced, most prominently in cocaine animals (Table 4.3; session $F_{2,18} = 18.768$, $p < 0.001$; session x drug- $F_{2,18} = 3.272$, $p = 0.061$; post-hoc paired samples t-test: cocaine: Day 1 vs Day 15- $t(7) = 6.365$, $p = 0.00038$; Day 1 vs Day 30- $t(7) = 5.469$, $p = 0.001$; saline $t < 2.053$, $p > 0.109$). Although visual inspection of the data shows cocaine animals appear to be more disinhibited as measured by premature responses, this was not significant (Table 4.3; session - $F_{2,18} = 3.038$, $p = 0.073$; risk-preference- $F_{1,9} = 7.510$, $p = 0.023$; remaining $F < 0.509$, $p > 0.609$). Omitted responses and collection latencies were unaffected during withdrawal (Table 4.3; $F > 2.630$, $p > 0.100$).

4.4 Discussion

These data show for the first time that experience with salient win-paired audiovisual cues may facilitate acquisition of cocaine self-administration, and precipitates greater sensitivity to decision-making deficits brought on by drug experience. As expected from our previous work, decision making as assessed by the risk-preference average was considerably more risky compared to historical data using the uncued rGT, and over 50% of animals exhibited a preference for the disadvantageous options on the crGT. Replicating our findings from Chapter 3, choice of the risky options was further exacerbated in these risk-preferring rats by cocaine self-administration. In contrast to the first experiment, crGT optimal decision-makers, despite exhibiting an advantageous decision-making profile at baseline, were statistically indistinguishable from risk-preferring rats in terms of their response to cocaine self-administration; their decision making also became more risky, such that advantageous decision making at baseline no longer protected them from the bias towards risky options induced by cocaine self-administration. As such, repeatedly making decisions under uncertainty for cue-

paired rewards may sensitize the choice deficits brought on by cocaine experience, even in individuals who are resilient to the risk-promoting effects of the cues themselves.

Surprisingly, all cocaine exposed rats, irrespective of decision-making phenotype, exhibited high levels of active lever pressing for and infusions of cocaine, most evident during the initial acquisition of self-administration. These rats did not show gradual increase in operant responding for drug commonly seen in the literature (Belin-Rauscent et al., 2016) or experiment 1, where lever pressing typically starts low and increases with repeated drug experience; instead, cocaine animals demonstrated robust responding for the drug-paired lever early in self-administration training. Although noteworthy, it is important to indicate self-administration procedures were limited to short access sessions on an FR1 schedule, which may not encapsulate compulsive drug seeking seen with extended access or second-order schedule of reinforcement paradigms (Arroyo et al., 1998; Wee et al., 2007). Future studies that incorporate these methods are required to determine if reward-paired cues sensitize habitual responding for drugs of abuse. It is also important to consider that although behavioural responding for drug appears to be greater in crGT rats compared to historic data within the lab and the field, there are several factors which can contribute to different acquisition profiles between studies and between research groups, including slight differences in training and housing methodologies. Additionally, the appearance of robust responding may simply be indicative of rapid learning of operant responses for reward, a process which cues may facilitate, although previous work in the lab suggests crGT trained rats do not learn the task faster than rGT counterparts (Barrus and Winstanley, 2016). Future studies should do a direct comparison of behaviourally naïve, rGT-, and crGT-trained rats' self-administration responses to determine how robust and reliable this effect is. Furthermore, adjudicating at which timepoint differences in responding for drug are

present (i.e. early versus late self-administration) would provide evidence as to whether crGT rats simply rapidly acquire self-administration or have prolonged potentiated responding to drug.

In contrast to our previous study (Ferland and Winstanley, 2016), all cocaine exposed animals demonstrated worsened decision making in diurnal crGT sessions run concomitantly with self-administration, as reflected by significant decrease in score for all rats, and exacerbated preference for P3 in risk-preferring animals. Similar to chapter three, we did not find any evidence that changes in choice were due to greater consumption of drug, in that the total number of infusions did not correlate with change in score. These data suggest worsened choice may be due to specific neuroadaptations in substrates underlying decision making after cocaine exposure, rather than neurotoxicity associated with drug use. One possible explanation for worsened decision making is that drug expectancy may have devalued sucrose as a reinforcer, as crGT sessions preceded self-administration. Indeed, previous data have shown devaluation of sugar prior to rGT performance resulted in increased choice of suboptimal options of the task (Zeeb and Winstanley, 2013). However, cocaine experienced animals did not show dampened motivation, as the number of omitted responses and trials completed were maintained during self-administration. Furthermore, collection latencies were decreased during this epoch, suggesting animals were motivated to consume rewards.

The mechanism by which cocaine influences decision making in the presence of reward-paired cues remains unclear. The shift in choice may indicate that the value of the risky options or associated cues was increased. Alternatively, cocaine experienced rats may have expressed a “myopia for the future” previously hypothesized by others (Bechara, 2005), in which the potential negative outcome associated with choosing risky options- in this case, the longer and more frequent penalties, and lower chances of reward- does not mitigate decision making. This

may be due to changes in dopamine receptor availability or sensitivity. Previous work on the crGT suggests involvement of the D₃ receptor in the decision-making process, as D₃ agonists increase disadvantageous choice while antagonists do the opposite on the crGT while having no effect on the uncued task variant (Barrus and Winstanley, 2016). Manipulations of D₃ activity can mediate responding for drug-paired cues. Indeed, D₃ blockade prevented the acquisition, expression and reinstatement of conditioned place preference (Vorel et al., 2002; Ashby et al., 2003; Hu et al., 2013), decreased drug seeking under a second-order schedule of cocaine reinforcement (Di Ciano, 2008), but left cocaine self-administration unaffected (Pilla et al., 1999). These data suggest D₃ activity may be particularly important in cue mediation of reward-response learning, but does not impact the value of the reward itself. Interestingly, two recent studies found D₃ occupancy (as measured by position emission tomography) was associated with psychostimulant abuse (Boileau et al., 2012; Le Foll et al., 2014) and less behavioural flexibility on a reversal learning task (Groman et al., 2016). Another study found antagonising the D₃ receptor can modulate activity within reward related circuitry in healthy controls and substance dependent individuals (Murphy et al., 2017). Perhaps cocaine experience increased activation of D₃ receptors, and resulted in potentiated choice of crGT options with the most salient cues. Future studies should incorporate modulation of D₃ activity in this model to investigate its role in cue-mediated decision making after cocaine exposure.

Interestingly, poor cognitive performance after cocaine experience was limited to the decision-making domain, as cocaine-exposed optimal rats showed a significant improvement of impulse control. These results are somewhat surprising, as impulsivity has been repeatedly implicated in drug addiction (Winstanley, 2011). Indeed, several studies have shown those with SUD have potentiated impulsive choice and behavioural disinhibition (Dalley and Robbins,

2017). In contrast to human studies, animal data have depicted a slightly more complex role for impulsivity in addiction. Indeed, high levels of impulsive choice and action have been associated with greater cocaine self-administration (Belin et al., 2008; Perry et al., 2008). However, other studies have shown cocaine experience actually improved prepotent responding in highly impulsive rats (Caprioli et al., 2013) and did not necessarily increase delay discounting in animals who prefer smaller-sooner versus larger-later rewards (Mitchell et al., 2014a). At the population level, risky choice and premature responding on the rGT are positively correlated, such that greater motor impulsivity is associated with higher levels of risky decision making (Barrus et al., 2015b). However, this relationship is not always apparent in each smaller, experimental cohort. In the current study, the level of premature responding we observed in optimal decision-makers self-administering cocaine was comparable to that seen in risk-preferring rats, and perhaps were more akin to highly impulsive rats seen in previous studies measuring behavioural disinhibition (Caprioli et al., 2013).

Surprisingly, the degree of impulsivity exhibited by risk-preferring rats was unchanged during the cocaine self-administration epoch, despite the marked increase in risky choice observed throughout this phase of the experiment. These data support the hypothesis that complimentary, but functionally distinct, neurobiological mechanisms subserving decision making and impulsivity may be variably affected by drug experience. A breadth of research has implicated mesocorticolimbic reward structures in mediating impulse control and decision making, including variable activity of the nucleus accumbens and prefrontal cortex, (Bolla et al., 2003; Winstanley, 2007; Stopper and Floresco, 2011; St Onge et al., 2012; Zeeb and Winstanley, 2013; Hart et al., 2015). However, manipulation of these regions results in divergent changes in cognitive performance. For example, selective lesions of the orbitofrontal cortex increased motor

impulsivity on the five-choice serial reaction time task, but decreased impulsive choice as measured by delay discounting, and left rGT performance intact (Winstanley et al., 2004; Winstanley, 2007; Zeeb and Winstanley, 2011). Similarly, lesioning the NAc after task acquisition resulted in behavioural disinhibition (Robbins, 2002), yet left rGT performance unaffected (Hosking et al., unpublished observations). Furthermore, variable connectivity to and from these regions would significantly affect expression of these distinct cognitive functions. Therefore, reward-paired cues may recruit distinct circuits in optimal versus risk-preferring rats, resulting in differential sensitivity to cocaine and drug-induced cognitive deficits. However, this is speculative, and further studies are required to probe the specific circuitries that delineate decision making from impulse control in response to drugs of abuse.

One potential variable not accounted for in this study is whether the cues used within the crGT are inherently stressful, conferring greater self-administration and sensitivity to stress-induced cognitive deficits. There is considerable research indicating activation of corticosterone is sufficient to increase self-administration (Zorrilla et al., 2014) and stress reactivity may play an important role in acquisition of drug seeking (Piazza et al., 1990). Furthermore, administration of corticosterone has been shown to attenuate acquisition of advantageous choice on a variant of the rGT (Koot et al., 2013). High levels of cocaine self-administration may also precipitate corticosterone release (Koob and Kreek, 2007), further affecting cognition. However, activation of the hypothalamic pituitary axis (HPA) has only been found in extended access studies, and rats in this study were restricted to limited access (Koob and Kreek, 2007). There is also no behavioural evidence to suggest the stimuli of the crGT are stressful as animals do not show avoidance of the most salient cues (Barrus and Winstanley, 2016), and unlike the previous rat gambling study, rats have more than 30 sessions of experience with these stimuli prior to drug

experience, and would have likely habituated to the cues as a stressor. Research has shown animals will habituate even to protocols that produce a robust stress response when repeatedly administered (McCarty, 2017). We did not measure corticosterone release or molecular adaptations of the HPA system, and believe it is unlikely these results are the product of enhanced stress. However, future studies incorporating a simple corticosterone assay after task exposure is would provide evidence to the role of stress reactivity in crGT rats.

Data from the 30-day withdrawal period revealed that, at least in optimal decision-makers, choice patterns can recover during abstinence, suggesting neuroadaptations that occur during the 24-hour drug-free period are not permanent in this subgroup. This pattern of recovery was not present in risk-preferring rats that had self-administered cocaine, supporting previous findings that cocaine experience results in long term alterations in decision-making circuitry within this group. Although preliminary, to our knowledge these are the first data of their kind to show an improvement in decision making during abstinence, suggesting optimal rats may be more resilient to cocaine-induced neuroadaptations. Visual inspection of the data suggests optimal animals renew a preference for P2 and decrease choice of P3, therefore reacquiring truly advantageous decision-making profile versus simple risk-aversion, which would have been reflected by increased choice of P1 (Zeeb et al., 2009). In addition to improvements in choice, cocaine optimal animals also exhibited greater number of trials completed, likely due to less time spent in punishing time outs associated with the risky options of the task.

Although not significant, optimal decision-makers and risk-preferring rats appeared to show differential changes in premature responding during withdrawal from cocaine self-administration. Risk-preferring rats demonstrated a similar behaviour to that seen in our previous rGT cohort (Ferland and Winstanley, 2016) and another study using the five-choice

serial reaction time task (Winstanley et al., 2009), in that premature responding transiently spiked two weeks into withdrawal but subsequently subsides. In contrast, although optimal decision-makers showed lower levels of motor impulsivity during self-administration, these levels increased after withdrawal, suggesting a double dissociation between the beneficial impact of abstinence on behavioural inhibition and decision-making performance. All cocaine-experienced rats showed decreased latencies to make decisions during withdrawal. This may be due to a general agitation of motor activity, as premature responses also appear to increase during this experimental epoch. However, the latency to collect rewards was unaffected in all animals, indicating the time taken to make a choice was specifically impacted. Although intriguing, these data are very preliminary and require subsequent replication to verify their validity. It would also be prudent for future studies to incorporate the incubation of craving paradigm to determine whether recovery of optimal decision making fosters resilience to “relapse”.

In addition to implications for SUD, these data may also shed crucial light on the foundation of gambling disorder (GD). GD is a psychiatric condition epitomized by risky decision making as the subject continues to play despite overwhelming financial loss (Clark et al., 2013; Petry et al., 2014). Like SUD, cues associated with monetary rewards elicit ventral striatum and insula activity (Sescousse et al., 2013; Limbrick-Oldfield et al., 2017), suggesting a common mechanism between craving for drugs and gambling. Electronic gaming machines utilize salient audiovisual cues to encourage play, and may be particularly highly addictive compared to other forms of gambling (Clark et al., 2013; Murch and Clark, 2015). Given the ability of experience with reward-paired cues to promote rapid acquisition of cocaine self-administration reported here, and to exacerbate elevated risky decision making following cocaine

self-administration noted here, experience with electronic gaming machines may potentially sensitize those with GD to other forms of addiction. Indeed, in a representative survey of pathological gamblers, 73% had comorbid alcohol use disorder and nearly 40% exhibited other drug abuse (Petry et al., 2005). Although both SUD and GD have several environmental and biological factors, the possibility that gambling devices may facilitate cross-sensitization of addiction is an important consideration when designing gaming machines, and demands further exploration

In sum, these data demonstrate for the first time that repeated exposure to win-paired cues and uncertain outcomes may be sufficient to facilitate operant responding for psychostimulants while simultaneously making the animal vulnerable to drug-induced cognitive deficits. These data are of importance for understanding how cue reactivity and decision making intersect to confer vulnerability to addiction, and provide a model to explore the neurobiological substrates contributing to a pro-addictive state.

4.5 Tables

Table 4-1 crGT performance prior to self-administration.

<i>Behavioural Measure</i>	<i>Saline Optimal</i>	<i>Saline Risk-preferring</i>	<i>Cocaine Optimal</i>	<i>Cocaine Risk-preferring</i>
Premature Responses	11.09 ± 2.69	30.04 ± 6.42	25.29 ± 7.23	29.59 ± 4.00
Trials Completed	129.34 ± 11.49	61.37 ± 6.54	94.47 ± 10.93	55.86 ± 3.38
Omitted Responses	0.22 ± 0.22	0.56 ± 0.39	0.61 ± 0.37	0.71 ± 0.35
Choice Latency	1.31 ± 0.19	1.65 ± 0.08	1.29 ± 0.43	2.35 ± 0.48
Collect Latency	1.09 ± 0.13	0.76 ± 0.19	0.98 ± 0.17	0.60 ± 0.05

Values presented are averages from the final three baseline sessions ± standard error of the mean (SEM).

<i>Behavioural Measure</i>	<i>Timepoint</i>	<i>Saline optimal</i>	<i>Saline risk-prefering</i>	<i>Cocaine optimal</i>	<i>Cocaine risk-prefering</i>
Trials Completed	Day 1	132.20 ± 8.75	67.74 ± 10.25	98.17 ± 10.20	62.02 ± 6.49
	Day 6	115.82 ± 19.23	64.02 ± 8.67	78.02 ± 12.78	50.54 ± 4.77
	Day 10	128.80 ± 13.07	69.60 ± 7.06	92.51 ± 16.93	55.54 ± 3.77
Omitted Responses	Day 1	0.40 ± 0.24	0.86 ± 0.46	1.00 ± 0.68	2.38 ± 1.38
	Day 6	1.00 ± 0.77	1.43 ± 0.69	1.00 ± 0.63	2.88 ± 1.26
	Day 10	0.20 ± 0.20	2.57 ± 1.95	1.50 ± 1.15	2.75 ± 1.16
Choice Latency	Day 1	1.25 ± 0.30	1.93 ± 0.30	1.24 ± 0.39	2.55 ± 0.58
	Day 6	1.82 ± 0.47	2.45 ± 0.47	1.66 ± 0.30	2.61 ± 0.59
	Day 10	1.74 ± 0.45	1.97 ± 0.33	2.05 ± 0.30	2.75 ± 0.56
Collect Latency	Day 1	1.04 ± 0.06	0.81 ± 0.21	1.12 ± 0.30	0.58 ± 0.04
	Day 6	1.06 ± 0.08	0.72 ± 0.25	0.79 ± 0.09	0.58 ± 0.08
	Day 10	1.03 ± 0.05	0.60 ± 0.08	0.72 ± 0.06	0.53 ± 0.06

Table 4-2 crGT performance during self-administration.

Values presented are averages per self-administration timepoint ± SEM.

<i>Behavioural Measure</i>	<i>Timepoint</i>	<i>Saline optimal</i>	<i>Saline risk-preferring</i>	<i>Cocaine optimal</i>	<i>Cocaine risk-preferring</i>
Score	Day 1	39.89 ± 56.37	-16.85 ± 36.70	-42.92 ± 30.12	-3.62 ± 16.95
	Day 15	29.46 ± 67.92	-25.10 ± 31.53	26.73 ± 6.25	-69.42 ± 23.73
	Day 30	36.58 ± 59.83	-40.27 ± 24.86	54.89 ± 4.03	-73.15 ± 21.19
Premature Responses	Day 1	4.35 ± 2.08	16.05 ± 6.39	4.53 ± 2.90	21.50 ± 2.45
	Day 15	11.83 ± 9.47	18.28 ± 4.84	12.83 ± 8.36	28.40 ± 4.13
	Day 30	4.85 ± 3.70	16.12 ± 5.73	11.92 ± 7.44	21.84 ± 4.29
Trials Completed	Day 1	123.00 ± 37.00	76.70 ± 13.19	95.67 ± 19.22	58.02 ± 3.53
	Day 15	108.00 ± 25.00	67.40 ± 6.95	113.67 ± 16.70	57.44 ± 3.62
	Day 30	128.50 ± 42.50	66.67 ± 7.69	117.33 ± 17.33	56.66 ± 4.19
Omitted Responses	Day 1	0.5 ± 0.5	4.67 ± 4.67	2.00 ± 1.53	1.00 ± 0.45
	Day 15	0.5 ± 0.5	4.00 ± 3.51	0.33 ± 0.33	0.20 ± 0.20
	Day 30	2.5 ± 2.5	2.33 ± 1.86	0.00 ± 0.00	0.60 ± 0.60
Choice Latency	Day 1	2.07 ± 1.26	2.11 ± 0.76	2.32 ± 0.21	1.84 ± 0.22
	Day 15	1.60 ± 0.80	1.89 ± 0.54	1.29 ± 0.45	1.13 ± 0.13
	Day 30	1.63 ± 1.32	1.80 ± 0.52	1.38 ± 0.57	1.02 ± 0.12
Collect Latency	Day 1	0.86 ± 0.27	1.26 ± 0.62	0.77 ± 0.10	0.49 ± 0.06
	Day 15	0.75 ± 0.28	1.38 ± 0.81	0.84 ± 0.04	0.52 ± 0.08
	Day 30	0.74 ± 0.31	1.40 ± 0.84	0.84 ± 0.06	0.49 ± 0.05

Table 4-3 crGT performance during 30 days of withdrawal from cocaine.

Values presented are averages per withdrawal timepoint ± SEM.

4.6 Figures

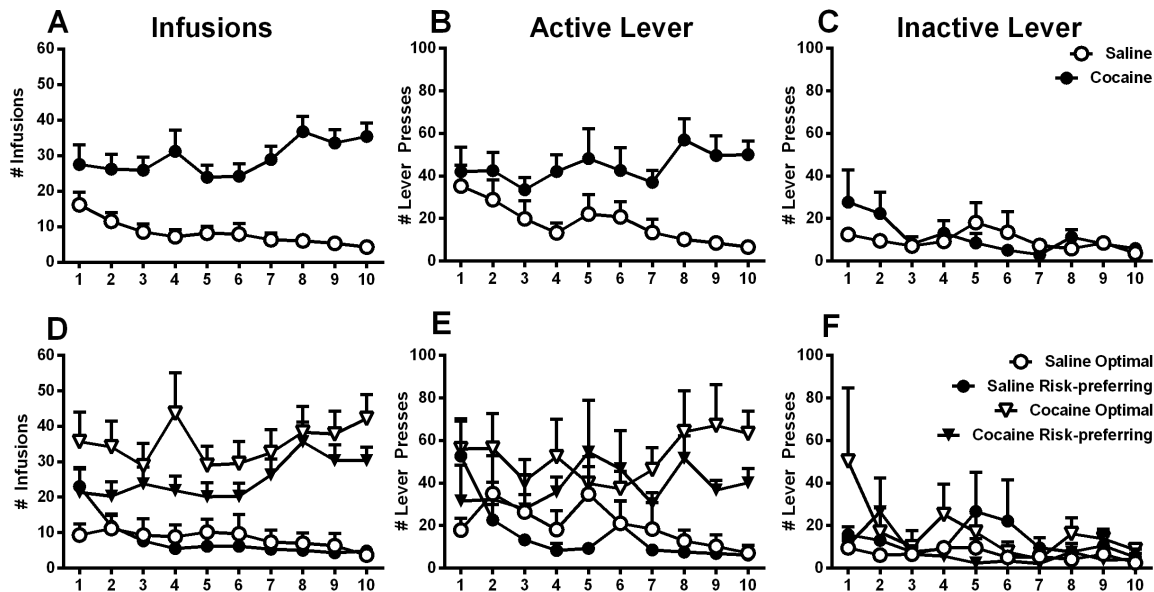


Figure 4-1 Responding during 3 hr drug self-administration sessions.

(A-B) Number of infusions and active lever presses for saline and cocaine animals over the self-administration period. Cocaine animals demonstrated significantly greater responding for drug, although this did not potentiate over the 10-day period. Saline rats decreased active lever responding over the 10 sessions. (D-E) Total number of active lever presses and infusions across self-administration by drug and risk-preference group. There were no significant differences by risk-preference in responding for drug. (C & F) Inactive lever pressing by drug and by decision-making phenotype. Responding for this lever remained low throughout the self-administration epoch and did not differ by risk-preference or drug group. Data are presented as mean \pm SEM.

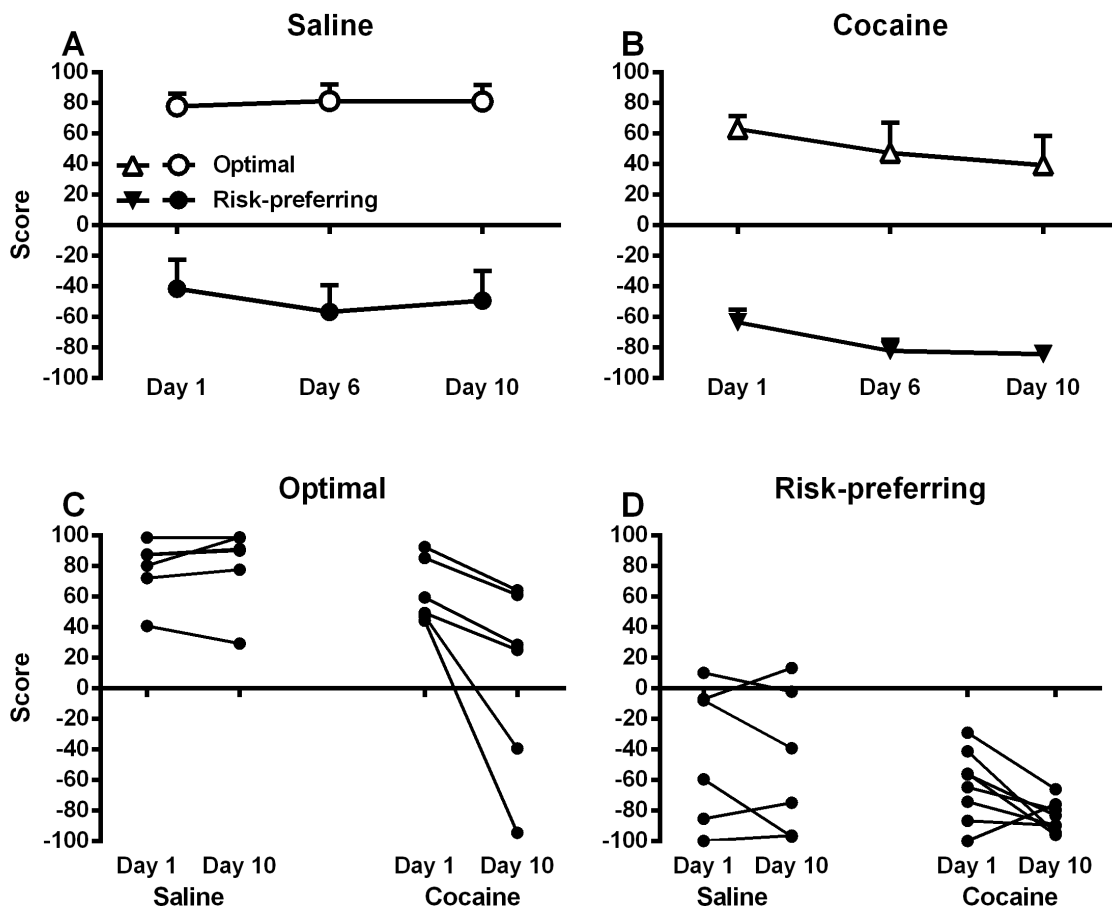


Figure 4-2 Changes in score over the self-administration period.

(A) Saline animals' score was unaffected by self-administration. (B) In contrast, all cocaine animals, regardless of risk-preference, exhibited significant decreases in score, reflecting an increase in disadvantageous choice after cocaine exposure. (C-D) Changes in individual rat score from day 1 to day 10 of self-administration in optimal and risk-preferring subgroups. Not only cocaine exposed animals exhibit decreases in score. Values in A & B are averages \pm SEM. Data points in C & D represent individual rats' score.

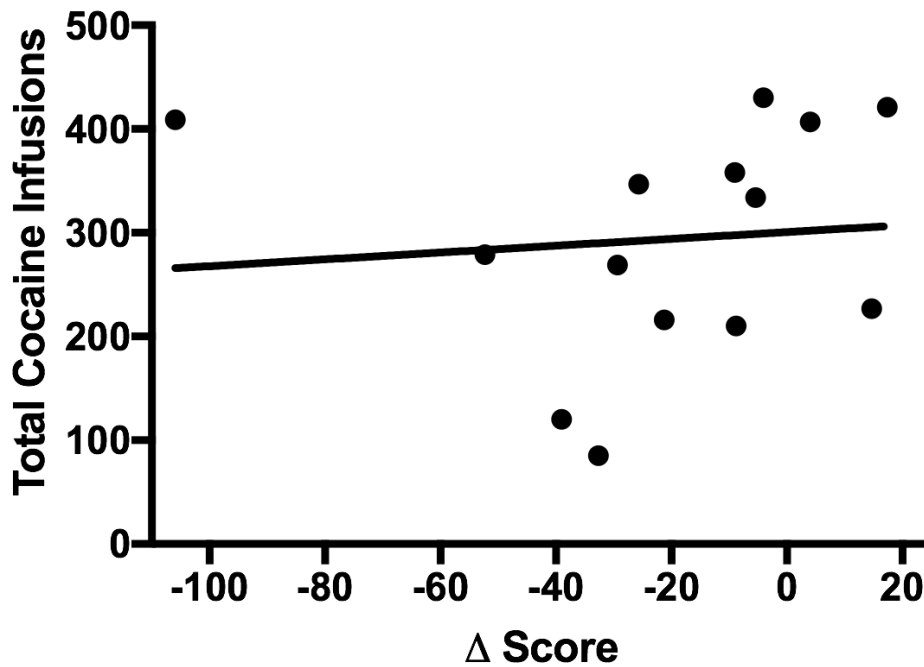


Figure 4-3 Correlation between number of cocaine infusions and changes in score on the crGT.

Despite similar levels of infusions received, cocaine animals' drug intake did not significantly correlate with worsened decision making, indicating changes in choice are likely due to neuroadaptations in reward circuitry underlying decision making rather than neurotoxicity after cocaine exposure. Data presented are individual cocaine rats.

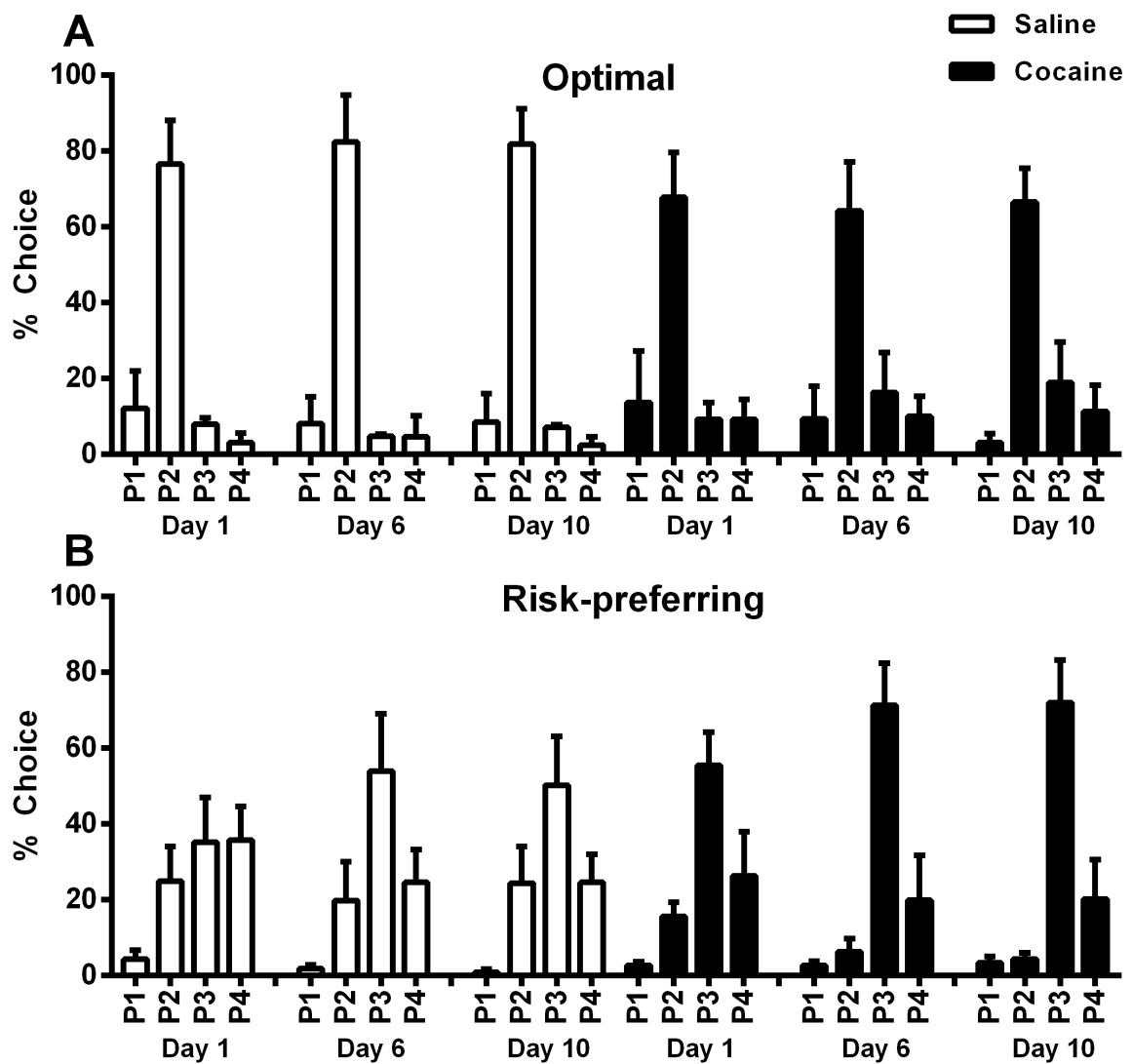


Figure 4-4 Individual choice preference during the 10-day self-administration period.

(A) In optimal animals, cocaine animals showed shifts in decision making across sessions, although not for an individual choice option. (B) Cocaine risk-preferring rats showed significant increases in P3, the most preferred option, and decreases in choice of P2, compared to saline counterparts. Values are averages \pm SEM.

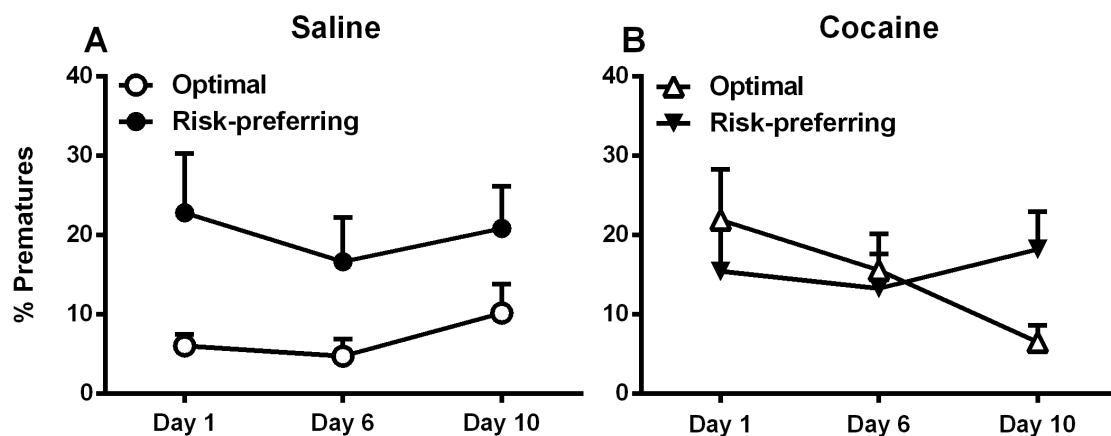


Figure 4-5 Premature responding on the crGT during the self-administration epoch.

(A) As with choice, impulsivity was unchanged in saline exposed animals. (B) Interestingly, cocaine-exposed optimal rats, but not risk-preferring, showed marked improvements in impulse control, suggesting drug-induced cognitive deficits were limited to decision making. Values are mean \pm SEM.

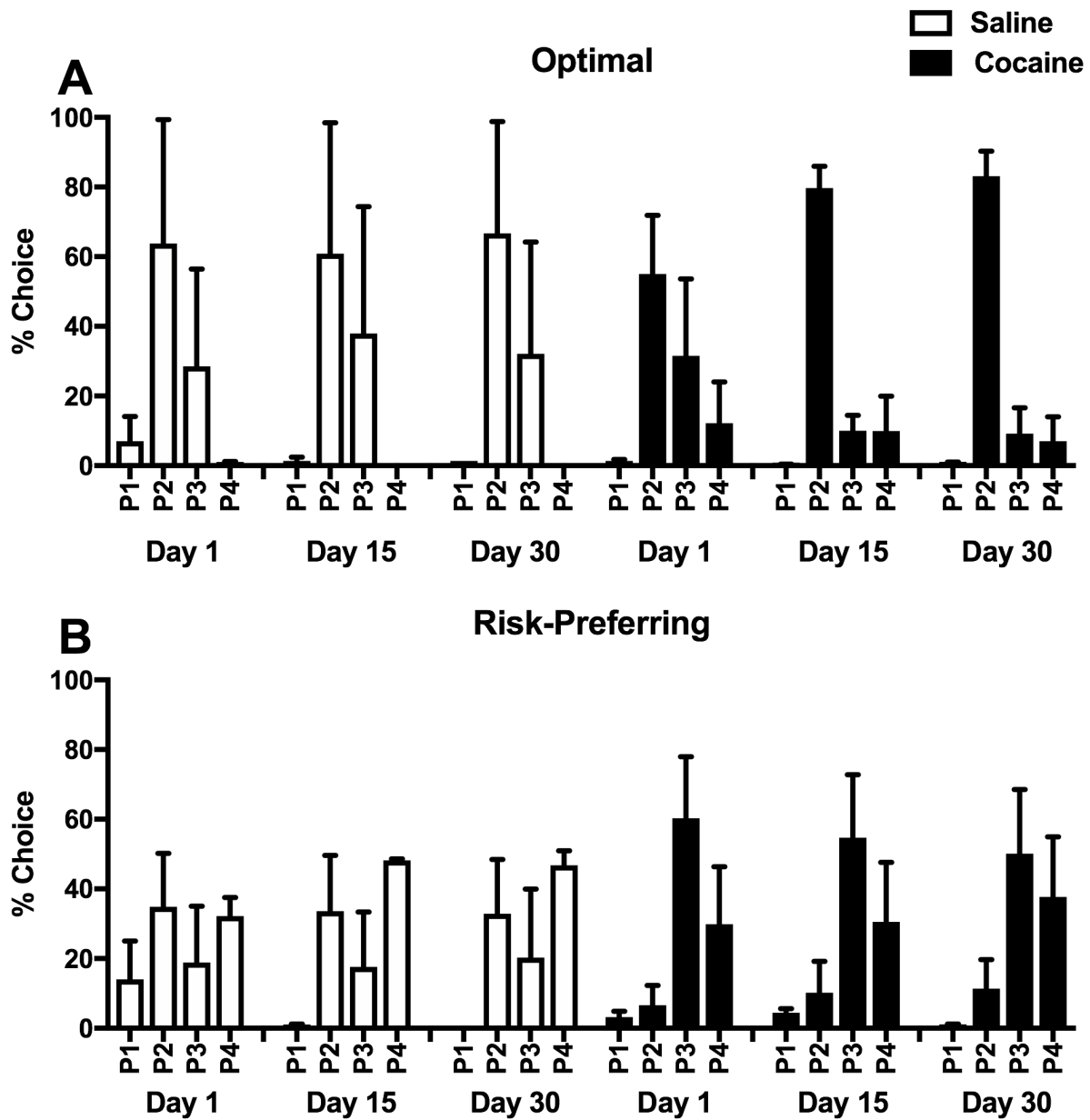


Figure 4-6 Individual choice preference during the 30-day withdrawal period.

(A) Although cocaine worsened decision making in optimal rats during self-administration, deficits in choice were somewhat recovered during withdrawal. (B) In contrast, cocaine-exposed risk-preferring rats maintained worsened performance during withdrawal, suggesting these animals are more susceptible to drug-induced neurobiological changes. Values are mean \pm SEM.

Chapter 5: Experience with the cued rat gambling task blunts dopamine efflux within the nucleus accumbens, fostering a reward deficient state.

5.1 Introduction

Uncertainty is a prominent element of everyday decision making, present in choices ranging from what to eat, to determining which job to take for career advancement. Therefore, being able to discern the most beneficial outcome from a variety of options is a key skill necessary for navigating common challenges. Hence, inflexible or aberrant choice can be particularly maladaptive, and has been implicated in a variety of psychiatric disorders including addiction (Bechara et al., 2001; Bolla et al., 2005; Verdejo-Garcia et al., 2007), and may play a crucial role in the cultivation and precipitation of the addicted state (Bechara, 2003; Stevens et al., 2013; Wang et al., 2013). Those who are raised in environments with greater levels of uncertainty, such as low socioeconomic backgrounds that are highlighted by uncertain income and resource availability, are at greater risk for the development of addiction (Degenhardt et al., 2016) and show more risk-prone behaviours (Ursache and Raver, 2015). Understanding how regular experience with, and choice under, uncertainty influences neural substrates involved in reward would provide significant insight as to how decision making and drug seeking intersect at a biological level.

Data from preclinical work shows risky decision making on a rat gambling task (rGT; i.e. preference for P3 and P4, outcomes associated with large but improbable wins) may precede addiction vulnerability— this subgroup of animals shows elevated operant responding for

cocaine, and has exacerbated disadvantageous choice after drug experience (Ferland and Winstanley, 2016). Another study found animals who were risk-prone in adolescence also exhibited greater cocaine self-administration and elevated risky choice in the presence of shock (Mitchell et al., 2014b). These data suggest that those who are more sensitive to risky outcomes may be particularly susceptible to the plastic changes within reward mechanisms after drug experience. Interestingly, animals trained on a cued variant of the rGT, the cued rat gambling task (crGT), showed similar propensity to seek drugs and worsened task performance after cocaine self-administration, regardless of decision-making phenotype (see chapter 4). Collectively, these data indicate interaction with uncertain contingencies, either by pre-existing preference or when made salient by cues, may affect neural circuitry implicated in drug seeking.

Recent behavioural work has shown extensive experience with uncertain rewards (i.e. 50+ sessions of variable-ratio schedule of reinforcement training) potentiated the locomotor response to amphetamine compared to animals trained on a fixed-ratio paradigm (Singer et al., 2012). Similar results have been found with classical conditioning, that rats exposed to a cue associated with uncertain sucrose delivery (0.5 probability of reward) showed greater ambulation after amphetamine administration and locomotor sensitization (Zack et al., 2014). These data raise the possibility that regular exposure to uncertain outcomes and their cues is enough to increase sensitivity to psychostimulants. Addition of audiovisual stimuli to the crGT increased choice of the most uncertain option, P3, similar to behaviour seen in rGT risk-preferring rats (Barrus and Winstanley, 2016). Therefore, greater experience with the uncertainty of P3 and P4 may potentiate responding for psychostimulants and foster drug seeking.

Another prospect is that rGT animals which develop a risk-preferring profile and crGT rats are more likely to make operant responses for reward-paired stimuli, and when placed into

self-administration paradigms, develop rapid associations between drug cues and operant responses. Indeed, risk-preferring animals showed elevated lever pressing for cocaine and developed greater incubation of craving after withdrawal, an operant measure for valuation of drug-paired cues (Grimm et al., 2001; Epstein et al., 2006). Although we did not test incubation of craving, crGT rats did show rapid responding on the active lever during cocaine self-administration. Perhaps the cues within the crGT foster a “cross-sensitization” for other reward-paired stimuli. This is somewhat in keeping with the incentive sensitization theory of addiction (Robinson and Berridge, 2008), which states the “wanting” for drugs is elicited by stimuli previously associated with use, and these cues are sufficient to drive craving and seeking. Preclinical work supports this hypothesis-- sign-tracking animals, or rats that show appetitive responding for cues more than reward, also respond more for drug-paired stimuli (Flagel et al., 2009; Flagel et al., 2010) and conditioned reinforcement (CRf; (Robinson and Flagel, 2009)), a measure of willingness to work for conditioned cues (Fanselow and Wassum, 2015). Therefore, risk-preferring animals on the uncued rGT, and rats trained on the crGT, may simply exhibit greater responsivity to the drug-paired stimuli, rather than be more sensitive to the rewarding properties of cocaine itself.

Determining specific neurobiological substrates contributing to both decision making and addiction is essential to inform therapeutic development. A study investigating neural correlates of pathological gambling, a behavioural addiction, found problem gamblers had augmented activity of the ventral striatum during IGT performance (Brevers et al., 2016). In methamphetamine abusers, hyperactivation within the ventral striatum after cue exposure is associated with higher subjective cravings and greater relapse risk, two factors found to be associated with poor IGT performance (Wang et al., 2012; Li et al., 2015). Extensive research

has shown dopamine (DA) within the mesostriatal network may play a significant role in mediating risky behaviours, as studies have shown cocaine, conditioned cues, and unpredictable rewards elicit DA release within the ventral tegmental area and striatum (Schultz, 1998; Fiorillo et al., 2003; Willuhn et al., 2010; Schultz, 2016a). Furthermore, phasic DA signalling is differentially recruited within the nucleus accumbens (NAc) to cues associated with an individual's preferred option (i.e. risk-averse animals show greater DA release during presentation of the certain choice cue; (Sugam et al., 2012; Sugam et al., 2014)), and manipulation of DA within the NAc can bias choice behaviour (Saddoris et al., 2015; Zalocusky et al., 2016). It is possible repeated exposure to uncertain rewards, particularly when those rewards are accompanied by salient audiovisual cues, may sensitize the DA response within the NAc, potentiate the salience of high-risk yet highly rewarding outcomes, and subsequently affect responding to drugs of abuse like cocaine.

To investigate how risky decision making, in the presence or absence of reward-concurrent cues, affected sensitivity to cocaine, animals were trained on the rGT and crGT. Before and after task training, cocaine-induced locomotor activity was measured to determine whether risk-preference or task experience sensitized the ambulatory response to drug. Following the final locomotor session, the degree to which animals were willing to learn a novel operant response that was reinforced solely by a classically conditioned stimulus was determined in a classic test of sensitivity to CRf. We were therefore able to determine whether baseline levels of risk-preference, or experience with salient reward-paired stimuli, influenced operant responding for reward-paired cues. Finally, we measured basal and cocaine-induced DA release within the NAc using microdialysis. Given previous results, we hypothesized that risk-preferring rats would show enhanced cocaine-induced locomotor activity, greater responding for conditioned stimuli,

and elevated NAc DA release, and that this would be particularly pronounced in animals trained on the crGT. We also hypothesised that the process of training rats on the rGT/crGT would exacerbate any enhanced sensitivity to the locomotor-stimulant properties of cocaine.

5.2 Additional Methods

Subjects

Thirty-two male Long-Evans rats were obtained from a lab breeding program for transgenic animals that express cre recombinase (Cre) in neurons that contain tyrosine hydroxylase (TH:Cre rats from Rat Resource and Research Centre, RRRC, Columbia, MO; Long-Evans-Tg(TH-Cre)3.1Deis, RRRC #00659; wildtype rats obtained from Charles River, St. Constant, Canada; (Witten et al., 2011)). Transgene status was determined as previously described (see general methods; n=16 per transgene, TH:Cre^{+/+}, ^{-/-}), but was not used for any experimental manipulation. Rats were weaned at post-natal day 21, and housed in same sex groups of two to three animals per cage. Animals had access to *ad libitum* standard rat chow and water until an average weight of approximately 300 g was reached. Rats were then transferred to the main vivarium at post-natal day 90, food-restricted to 85% of their free-feeding weight, and maintained in colony conditions previously described (see general methods).

Apparatus

Locomotor testing was completed in 40 cm² plexiglass boxes fitted with video cameras and ambulatory activity was counted using behavioural tracking software (Ethovision 3.1, Noldus). CRf testing was completed in operant boxes identical to those used for the gambling

tasks within a separate room in the facility to control for task interference. CRf testing utilized levers and cue lights situated on either side of the food magazine. For microdialysis sessions, rats were placed in a 40 cm³ plexiglass box fitted with a swivel to the sampling apparatus (see microdialysis subsection).

Locomotor activity

Locomotor testing was completed prior to and after gambling task training. As in previous studies (Singer et al., 2012; Zack et al., 2014), animals were allowed a 1 hr habituation period to the locomotor chamber, after which they were given a 1 ml/kg i.p. injection of saline. After 1 hr of locomotor recording, animals were then given a 10 mg/kg i.p. injection of cocaine, after which recording resumed for 1 hr. Total distance travelled (cm) was calculated using tracking software and parsed into 5-min bin sums for analyses.

Conditioned Reinforcement

CRf testing was completed after the final locomotor sessions had concluded. During ten 1 hr conditioning sessions, animals were presented with two cue lights. One light (CS+) was paired with delivery of a sugar pellet reward, while the other light (CS-) did not predict reward. The location of the CS+/- (left or right of the food tray) was counter-balanced across the cohort. On day 11, two levers were presented for 1 hr. Responses on one lever would result in delivery of the CS+, and the other the CS-, but no sugar was delivered at any time. The number of responses for the CS+ and CS- were recorded and calculated as a ratio of the total number of active lever responses/ sum of responses on both levers.

Surgeries

For microdialysis, twenty-eight animals underwent aseptic stereotaxic surgery. Rats were anesthetised with isoflurane (5% induction, 2-3% maintenance), and bupivacaine was administered at the surgical site as a local anesthetic. Animals were bilaterally implanted with 15 mm 19G nitric acid passivated stainless-steel guide cannulae above the NAc (from bregma +1.7 mm anterior and \pm 1.1 mm lateral; from dura -1.0 mm ventral; (Paxinos and Watson, 1998)), secured via skull screws and dental cement. Stainless steel obturators (15 mm) maintained patency of the guides until probe implantation. Remaining animals (n=4) were used for subsequent breeding.

Microdialysis

Microdialysis probes were constructed from Filtral 12 AN69HF semi-permeable hollow fibres (2 mm long, 340 μ m OD \times 4 mm, 65 kDa molecular weight cut-off; Hospal, Germany) and silica inlet-outlet lines (75/150 μ m ID/OD). The day prior to microdialysis experiments, probes were flushed with artificial cerebrospinal fluid (aCSF) (10.0 mM sodium phosphate buffer with 147.0 mM NaCl, 3.0 mM KCl, 1.0 mM MgCl₂ and 1.2 mM CaCl₂; pH 7.4) and inserted via guide cannulae (dialysis membrane spanned -4.8 to -6.8 mm ventrally). Rats remained in the testing chamber overnight (14–16 h) with continuous perfusion of aCSF at 1.1 μ l/min with available food and water. In the morning, dialysates were collected at 10-min intervals and assayed for DA. Once a stable baseline was established (<10% fluctuation over four consecutive samples, approximately 8 baseline samples taken per rat), animals were administered a 1 ml/kg i.p. injection of saline followed by a 10 mg/kg injection of cocaine. Dialysates were collected for 1

hr after each injection. This process was repeated for the opposite hemisphere two to four days later to prevent sensitization to cocaine and allow the DA system to recover. The first hemisphere sampled was counterbalanced across animals. After the experiment, animals were sacrificed by live decapitation, brains were sectioned at -20° C on a cryostat, and sections were stained with cresyl violet. Probe placements were histologically verified following the experiment.

HPLC

Samples were analysed via high-performance liquid chromatography (HPLC) with electrochemical detection. HPLC systems were composed of the following: an ESA 582 pump (Bedford, USA), a pulse damper (Scientific Systems, USA), an inert manual injector (Rheodyne, USA), a Super ODS TSK column (Tosoh Bioscience, USA) and an Intro Electrochemical detector (Antec Leyden, The Netherlands). The mobile phase [70 mM sodium acetate buffer, 40 mg/l EDTA and 6 mg/l sodium dodecyl sulfate (adjustable); pH 4.0, 10% methanol] flowed through the system at 0.15 ml/min. EZChrome Elite software (Scientific Software, USA) was used to acquire and analyse chromatographic data.

Statistical Analyses

All data were analysed using SPSS statistical software (version 24.0, IBM). Rat gambling task data was analysed as described previously (see general methods). To ensure transgene status of animals did not account for any difference in behaviour, separate analyses were conducted for all rGT and crGT variables. The total responses on each lever during the CRf probe session were subject to repeated-measures ANOVA (two levels- active, inactive). CRf ratio data were also

subjected to a univariate ANOVA. For locomotor activity, total distance travelled (cm) was analyzed by repeated-measures ANOVA with treatment (two levels- saline, cocaine) and time bin (6 levels: 6 x 5 min bins) as within-subjects factors. Only the first 30 min of behaviour were analysed to capture locomotor counts while cocaine was onboard (Ciccarone, 2011). Task (rGT, crGT, n = 16 per task), and risk-preference (optimal, risk-preferring, n=16 per risk-preference) were included as between-subjects factors for locomotor and CRf analyses.

Averaged DA dialysate concentrations collected from each hemisphere by microdialysis were subjected to a repeated-measures ANOVA (treatment: 3 levels- baseline, saline, cocaine; bin: 6 levels- 6 x 10 min bins). Four rats had a substituted baseline value from an earlier measurement for one hemisphere due to unusual deviation from an otherwise stable baseline (i.e. 3 out of 4 baseline values had < 10% variability). Saline and cocaine neurochemical data were transformed into percentage of change over baseline, and were analysed with a repeated-measures ANOVA (5 levels, 5 x 10 min bins after drug was on board). Five animals were excluded from microdialysis analyses due to improper probe placement, and one animal's data was excluded due to illness. Analysis of DA efflux included between-subjects factor task (two levels, n=11 rGT, n=11 crGT) but not risk-preference due to lack of individual differences seen in locomotor data (see results).

5.3 Results

Rat gambling task performance

Rats trained on the rGT and crGT exhibited similar behavioural profiles to previous cohorts, with crGT animals showing greater preference for P3, lower score, greater premature

responding, and faster reward collection latencies (Table 5.1; choice- $F_{3,72} = 8.063$, $p < 0.001$; choice x risk-preference- $F_{3,71} = 8.502$, $p < 0.001$; choice x task- $F_{3,72} = 2.619$, $p = 0.066$; choice: risk-preference- $F_{1,28} = 4.584$, $p = 0.041$, risk-preference x task- $F_{1,28} = 6.215$, $p = 0.019$; score: risk-preference- $F_{1,28} = 57.484$, $p < 0.001$; task- $F_{1,28} = 8.928$, $p = 0.006$; premature responding: task- $F_{1,28} = 5.649$, $p = 0.025$; omissions: risk-preference- $F_{1,28} = 11.703$, $p = 0.002$; trials: task- $F_{1,28} = 4.114$, $p = 0.052$; collection latencies: task- $F_{1,28} = 7.643$, $p = 0.010$; all remaining $F < 2.574$, $p > 0.120$). rGT and crGT performance was indistinguishable between TH:Cre^{+/-} and ^{-/-} rats ($F < 1.912$, $p > 0.178$). Therefore, transgene status was excluded as a between-subjects measure for all remaining analyses.

Locomotor testing

Analysis of locomotor activity revealed, surprisingly, that rGT trained animals showed a significant increase in cocaine-induced locomotor activity compared to crGT rats after training (Figure 5.1A; day- $F_{1,28} = 5.649$, $p = 0.025$; day x bin- $F_{5,140} = 3.410$, $p = 0.006$; day x bin x task- $F_{5,140} = 2.794$, $p = 0.019$). All animals showed a significant increase in ambulation after cocaine (treatment- $F_{1,28} = 36.108$, $p < 0.001$; treatment x bin- $F_{5,140} = 7.340$, $p < 0.001$; treatment x task, treatment x risk-preference- $F < 0.316$, $p > 0.579$), but post-hoc analyses showed a significant increase in locomotor counts across days for rGT, but not crGT, trained rats (Figure 5.1B; rGT: day- $F_{1,14} = 6.068$, $p = 0.027$, treatment $F_{1,14} = 20.577$, $p = 0.0005$; day x bin- $F_{5,70} = 2.956$, $p = 0.018$; treatment x bin- $F_{5,64} = 2.957$, $p = 0.022$; rGT cocaine only: Day- $F_{1,14} = 4.781$, $p = 0.046$; crGT: day- $F_{1,14} = 0.370$, $p = 0.553$; treatment- $F_{1,14} = 15.672$, $p = 0.001$; treatment x bin- $F_{5,70} = 6.191$, $p < 0.001$). Neither rGT or crGT animals showed changes in locomotor activity after

saline (Figure 5.1D-F; day- $F_s < 0.529$, $p > 0.479$), suggesting crGT experience prevented sensitization to cocaine rather than suppressing locomotor activity in general.

There were subtle differences in ambulatory activity before and after training between optimal decision-makers and risk-preferring rats, most prominently in animals trained on the crGT (Fig. 5.1C; day x bin x task x risk-preference- $F_{5,140} = 2.270$, $p = 0.051$; crGT only: day x bin- $F_{5,70} = 3.239$, $p = 0.011$; day x bin x risk-preference- $F_{5,70} = 2.992$, $p = 0.017$; treatment x bin x risk-preference- $F_{5,70} = 2.517$, $p = 0.04$). More specifically, optimal decision-makers on the crGT tended to be slightly more active than risk-preferring rats following the first administration of cocaine, prior to training (crGT cocaine- bin- $F_{5,70} = 5.744$, $p < 0.001$; day x bin- $F_{5,70} = 2.682$, $p = 0.028$; bin x risk-preference- $F_{5,70} = 2.21$, $p = 0.06$). Also, crGT risk-preferring animals showed slightly blunted locomotor activity following saline injections before training, but increased these counts after task experience (Figure 5.1F; crGT saline: day x bin- $F_{5,70} = 2.399$, $p = 0.046$; day x bin x risk-preference- $F_{5,70} = 2.609$, $p = 0.03$; other interactions $F < 2.048$, $p > 0.100$; crGT risk-preferring saline: day x bin- $F_{5,55} = 2.373$, $p = 0.051$; day 1 saline: risk-preference- $F_{1,14} = 5.030$, $p = 0.042$; day 2 saline: risk-preference- $F_{1,14} = 0.253$, $p = 0.623$). This suggests that cues may make risk-preferring animals more sensitive to i.p injections, although this did not affect their responding for cocaine.

Risk-preference did not significantly account for the sensitization effect seen in rGT animals (Figure 5.1B; day x risk-preference, day x bin x risk-preference, risk-preference $F_s < 1.094$, $p > 0.372$), although variable responding after cocaine exposure was present between the risk-preference groups, and is visibly albeit not significantly lower in risk-preferring animals prior to training (rGT rats: bin x risk-preference- $F_{4,55}$, $p = 0.08$). For all analyses, no other significant interactions were present by day, treatment, task, or risk-preference ($F < 2.856$, $p >$

0.102). From these data it would seem that, although risk-preferring rats do show slightly blunted basal locomotor activity, these rats are not more or less sensitive to cocaine after training. Therefore, regular interaction with salient win-paired cues, more than risk-preference, mitigated the locomotor response to cocaine.

Conditioned reinforcement

All animals expressed CRf as indicated by a preference for the lever associated with the CS+ (Figure 5.2A; lever- $F_{1,21} = 9.409$, $p = 0.006$). However, there were no significant differences by task or by risk-preference for lever chosen or ratio of responding (Figure 5.2B-C; task- $F_{1,21} = 1.57$, $p = 0.224$; risk-preference- $F_{1,21} = 1.634$, $p = 0.215$; task x risk-preference- $F_{1,21} = 0.144$, $p = 0.709$). We also examined the latency to respond on either lever, and likewise found no significant difference between animals trained on the rGT/crGT or optimal decision-makers/risk-preferring rats (data not shown; $F < 2.111$, $p > 0.161$). These data suggest neither risk-preference nor task experience sensitized responding for CRf.

Dopamine microdialysis

Histological analyses found most animals had appropriate placement of microdialysis probes on the boundary of the NAc shell and core (see figure 5.3 for placements). As expected, cocaine significantly increased dopamine levels within the NAc compared to baseline and saline (Figure 5.4 A-B; treatment- $F_{1,24} = 76.839$, $p < 0.001$; bin- $F_{1,38} = 10.547$, $p < 0.001$; treatment x bin- $F_{2,36} = 6.909$, $p = 0.004$). However, crGT rats exhibited significantly less basal NAc DA efflux compared to rGT rats (task- $F_{1,20} = 4.598$, $p = 0.044$; treatment x task, bin x task, treatment x bin x task- $F < 1.064$, $p > 0.350$), a difference pronounced at baseline and after saline (baseline:

task- $F_{1,20} = 0.045$; bin- $F_{2,36} = 3.316$, $p = 0.05$; bin x task- $F_{2,36} = 2.739$, $p = 0.08$; saline: task- $F_{1,20} = 5.406$, $p = 0.031$; bin- $F_{5,93} = 3.588$, $p = 0.006$; bin x task- $F_{5,93} = 1.169$, $p = 0.330$).

Although crGT rats visibly appear to have less NAc DA efflux after cocaine, this was not significant (task- $F_{1,20} = 3.031$, $p = 0.097$; bin- $F_{2,35} = 8.365$, $p = 0.002$; bin x task- $F_{2,35} = 0.949$, $p = 0.385$). Interestingly, although crGT animals had less NAc DA efflux compared to rGT rats, these rats did show slightly greater percent increase after cocaine compared to uncued counterparts (Figure 5.4B; task- $F_{1,19} = 3.513$, $p = 0.076$; treatment- $F_{1,19} = 48.288$, $p = 0.0000013$; bin- $F_{2,33} = 8.909$, $p = 0.001$; treatment x bin- $F_{2,34} = 6.916$, $p = 0.004$; all interactions by task- $F < 2.947$, $p > 0.102$). Therefore, though crGT rats may have less basal NAc DA compared to rGT rats, release may be slightly more pronounced after cocaine exposure.

5.4 Discussion

This work shows unequivocally for the first time that experience with cue-enhanced decision making blocks sensitization to cocaine via lowered basal NAc DA release, which may in turn confer greater vulnerability to addiction. Indeed, crGT trained rats showed significantly less basal DA efflux within the NAc but comparable if not slightly greater release after cocaine. Additionally, crGT compared to rGT rats show less cocaine-induced ambulation after training. Interestingly, CRf responding was comparable regardless of task experience or risk-preference, indicating greater responding for rewards is likely due to a hypodopaminergic state rather than a robust operant response for reward-paired cues. Taken in context with the literature reflecting DA underactivity in addiction, these data show experience with such cues may precipitate both risky decision making and a pro-addictive phenotype via mesostriatal circuitry.

Recent studies found exposure to uncertain outcomes resulted in increased locomotor responding for amphetamine, signifying that regular interaction with uncertainty enhances the response to psychostimulants (Singer et al., 2012; Zack et al., 2014). While rGT trained animals exhibited a sensitized locomotor response to cocaine after training, crGT rats did not. These data are in keeping with the hypothesis that exposure to uncertain outcomes can foster a greater response to psychostimulants, but also offer an interesting argument that this is not necessarily a negative outcome. Preclinical work has drawn links between greater sensitivity to amphetamine-induced locomotion, both acutely and via behavioural sensitization, and rapid acquisition of psychostimulant self-administration (Piazza et al., 1989, 1990). However, another study showed neither acute nor sensitized amphetamine-induced ambulation was associated with greater self-administration, nor did it confer greater vulnerability to reinstatement of drug seeking (Sutton et al., 2000). This is not entirely surprising, as it is known self-administration does not equate to habitual drug seeking (Belin and Deroche-Gamonet, 2012), but rather reveals the drug is reinforcing. Also, although rGT optimal rats acquired self-administration, only risk-preferring animals showed heightened drug seeking and exacerbated decision-making deficits after cocaine exposure (Ferland and Winstanley, 2016), suggesting optimal rats may show a more “recreational” drug-taking phenotype. Therefore, the sensitization seen in rGT animals may reveal normal responding for drugs like cocaine, while the locomotor response in crGT rats may reflect vulnerability to drugs of abuse.

Microdialysis results showed crGT rats exhibited significantly less basal DA release within the NAc compared to rGT animals, and experience with this task has been associated with robust cocaine self-administration regardless of decision-making preference (Chapter 4). These results are somewhat consistent with the reward deficiency hypothesis of addiction, which states

SUD may be the result of a hypodopaminergic state, resulting in greater sensitivity to drugs of abuse (Blum et al., 2012a; Blum et al., 2012b). Indeed, cocaine dependent subjects showed blunted dopamine release within the ventral striatum after amphetamine administration, had lower ratings of euphoria after amphetamine, and greater choice to self-administer cocaine (Trifilieff et al., 2017). Likewise, crGT rats showed vigorous responding for cocaine self-administration. In slight contrast, crGT rats also showed slightly potentiated cocaine-induced DA release, but these animals only had limited, acute drug experience, whereas cocaine dependent subjects' blunted response to amphetamine may be the result of chronic psychostimulant administration. However, both results suggest low NAc DA release is associated with greater drug seeking. Low basal dopamine may also potentiate risky choice, as crGT experience results in greater choice of the disadvantageous options of the task. Indeed, DA fluctuations within the NAc have been associated with risk-preference (Sugam et al., 2012) and phasic release is recruited during probabilistic decision making (St Onge et al., 2012). However, to our knowledge, no preclinical model has investigated whether blunted NAc dopamine causes disadvantageous decision making. Subsequent studies that manipulate DA efflux within the NAc are required to confirm a causal relationship between blunted DA release and sub-optimal choice.

There are also some important limitations to the DA data collected in this study to note. Firstly, we only measured general NAc DA release, in contrast to investigating NAc subregions. This distinction is an important consideration, as previous work has shown the NAc shell and core recruit DA differently during distinct phases of decision making (Sackett et al., 2017), and cocaine-experienced animals show differential DA responses within each subregion during withdrawal (Saddoris et al., 2017). Future studies probing DA efflux within these subregions

would help us to delineate involvement of these areas in this effect. Secondly, although crGT rats have lowered basal release compared to rGT rats, we do not know if this is the result of reduced release after task experience, or simply an abrogating effect cues have on DA adaptations after behavioural training. Simply put, rGT rats may be showing a “normal” change in NAc DA efflux after behaviour, whereas crGT experience prevents these changes. rGT trained rats may also be receiving a greater number of sugar rewards due to advantageous task performance, which may contribute to greater DA release after training compared to crGT animals. Future studies should investigate DA efflux before and after task training to determine whether the crGT experienced animals have a decrease or simply no change in DA signalling after training. The inclusion of yoked control groups, in which the number of sugar pellets received is equal and/or cues are delivered independent of reward, would also provide evidence as to whether greater sugar rewards, the cues themselves, or experience with the contingencies of each task affects NAc DA release.

It is possible that repeated exposure to salient reward-paired cues combined with the uncertain outcomes produces large spikes in DA release, resulting in compensatory down-regulation of efflux but upregulation of postsynaptic receptors in the long term. Therefore, greater activity at receptors brought on by a powerful reward, such as cocaine, may potentially reinforce behaviour. Indeed, activity of the DA D₃ receptor, previously implicated in drug seeking under the guidance of cues (Di Ciano, 2008), enhanced risky decision making on the crGT (Barrus and Winstanley, 2016). Greater D₃ occupancy has also been associated with inflexible decision making (Groman et al., 2016). Furthermore, excitation of D₁ receptors within the basolateral amygdala, a region implicated in reward valuation and decision making on the rGT (Zeeb and Winstanley, 2011, 2013), enhanced choice of the uncertain reward on a

probabilistic discounting task in risk-averse animals, while D₂ agonists increased lose-shift behaviour in risk-prone rats (Larkin et al., 2016). Increased DA receptor activity has also been implicated in drug and behavioural addictions: greater D₂/D₃ receptor activation is associated with more subjective excitement in those with pathological gambling (Linnet et al., 2011), while those with SUD exhibit blunted D₂ receptor activity in abstinence (Volkow et al., 2009). Drug-paired cues have been shown to result in greater DA D₂ receptor occupancy (Volkow et al., 2006; Wong et al., 2006). In this study, we did not assess DA receptor expression between crGT and rGT rats. Future molecular studies are required to investigate how DA receptor expression, namely those in the D₂ family, is affected by crGT training.

Perhaps one of the most surprising results of this study was the lack of differences in responding for CRf. Many studies have shown appetitive cues and uncertainty can increase sign-tracking (Tomie et al., 2008; Robinson et al., 2014). Moreover, our previous results found risk-preferring animals in the rGT showed greater responding for drug-paired cues after withdrawal, suggesting a potential link between risky choice and cue responsivity (Ferland and Winstanley, 2016). However, the cues of the crGT in the absence of variable reward contingencies were not enough to elicit choice preference, and therefore on their own do not bias behaviour (Barrus and Winstanley, 2016). Another prospect is the CS+ implemented in CRf is neither novel nor variable enough to be rewarding for crGT trained animals, yet this is unlikely as all rats expressed significant responding for the CS+. Therefore, although behaviourally experienced animals showed responding for conditioned cues, it is unlikely this operant response drives risky choice on either the crGT or rGT.

This study also raises important questions as to how sensitivity to risk and experience with reward-paired cues may synergistically impact addiction susceptibility. A recent study

found adolescents with problem gambling were also prone to risky decision making (Ciccarelli et al., 2016). With the advent of increasingly available gambling opportunities (i.e. internet gambling) which often comes laden with salient cues, it is possible experience with such games may foster future addiction, particularly during crucial developmental periods. Subsequent studies using animal models are required to determine how exposure to salient win-paired cues, particularly early in life, may influence sensitivity to habitual drug seeking or gambling.

In conclusion, these data provide definitive evidence that salient audiovisual stimuli, when paired with uncertain reward, can foster a hypodopaminergic state, which in turn may result in greater responding for drugs of abuse. These results shed important light on the neural biological substrates which influence responding to reward-paired cues, decision making under uncertainty, and addiction risk.

5.5 Tables

Table	Behavioural Measure	<i>rGT</i>	<i>crGT</i>	<i>Optimal</i>	<i>Risk-preferring</i>
5-1	P1	7.61 ± 2.10	9.76 ± 2.91	10.42 ± 3.29	6.95 ± 1.39
	P2	62.72 ± 9.34	23.91 ± 5.04	66.33 ± 9.69	20.30 ± 3.69
	P3	13.81 ± 3.29	39.09 ± 7.43	8.10 ± 2.22	44.79 ± 7.45
	P4	15.86 ± 3.22	27.24 ± 5.48	15.14 ± 3.03	27.95 ± 5.57
	Score	40.67 ± 9.30	-32.66 ± 9.15	53.50 ± 8.61	-45.49 ± 7.78
	Premature Responses	12.09 ± 1.99	22.40 ± 3.31	14.85 ± 2.45	19.64 ± 3.10
	Trials Completed	89.80 ± 12.39	64.44 ± 8.69	93.99 ± 12.76	60.25 ± 7.89
	Omitted Responses	3.21 ± 0.66	1.21 ± 0.34	2.48 ± 0.52	1.94 ± 0.56
	Choice Latency	1.64 ± 0.23	1.29 ± 0.19	1.44 ± 0.21	1.49 ± 0.22
	Collect Latency	0.94 ± 0.13	0.61 ± 0.09	0.90 ± 0.12	0.65 ± 0.09

Baseline rGT and crGT performance.

Values are averages from final three baseline sessions ± SEM.

5.6 Figures

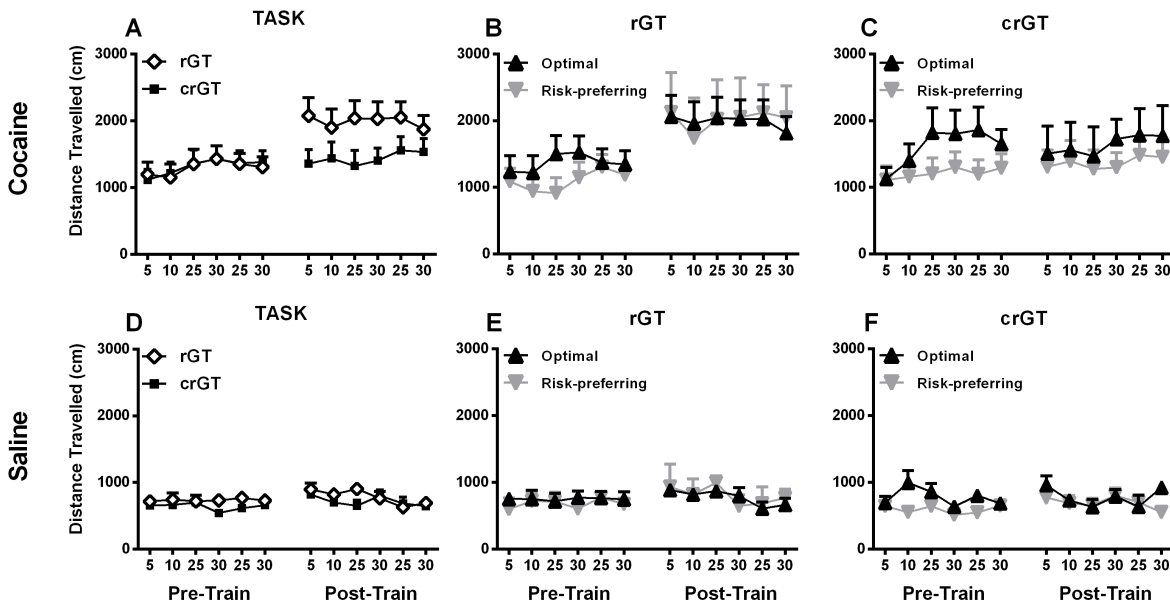


Figure 5-1 Locomotor activity pre- and post- task training after acute cocaine or saline administration.

(A & D) Prior to task training, animals exhibited robust ambulation after cocaine exposure compared to saline. However, after training, only rGT experienced rats (A-B) showed significant potentiated cocaine-induced locomotor activity, suggesting training on this task heightened the locomotor response to psychostimulants. (A & C) In contrast, crGT trained animals maintained comparable locomotor activity as prior to training, indicating a blunted response to cocaine as compared to uncued animals. (B, C) Risk-preference did not mitigate the response to cocaine after training, although risk-preferring animals showed blunted cocaine-induced ambulation prior to task experience as compared to optimal decision-makers. (D-F) Saline-induced locomotion remained relatively stable before and after task experience, indicating the potentiated response seen in rGT trained animals was unique to cocaine. Values are mean \pm SEM.

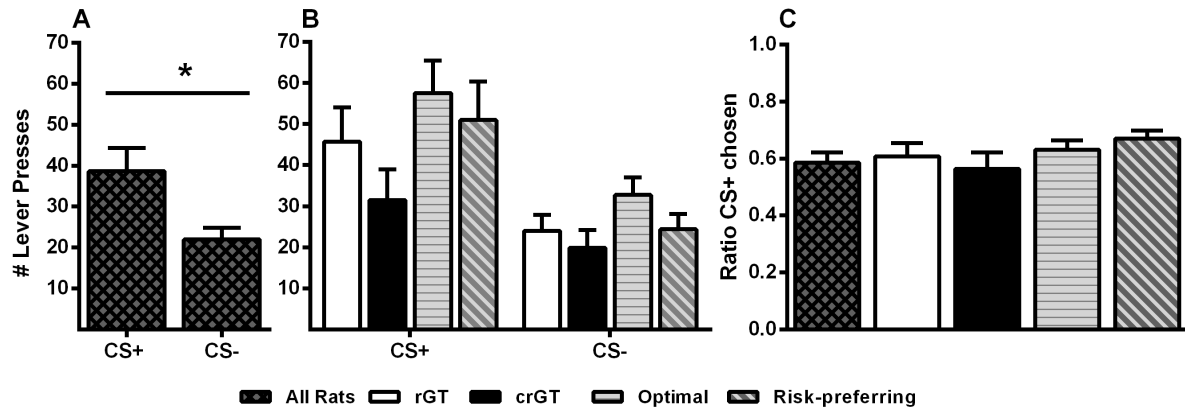


Figure 5-2 Responding during CRf test.

(A) All animals exhibited a significant preference for the CS+ lever compared to the CS-.

However, there were no significant differences by task experience or risk-preference, either for lever preference (B) or ratio for CS+ (C). These data suggest that although rats acquired the CRf response, experience with salient reward-paired cues or individual risk-preference did not mitigate operant responding for cues. Asterisk denotes $p < 0.05$. Values are averages \pm SEM.

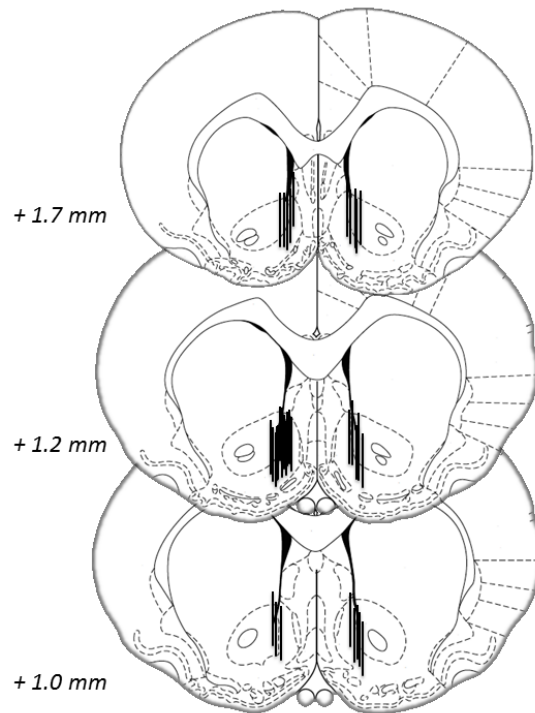


Figure 5-3 Placement of probes on the border of the NAc shell and core at specific anterior-posterior coordinates.

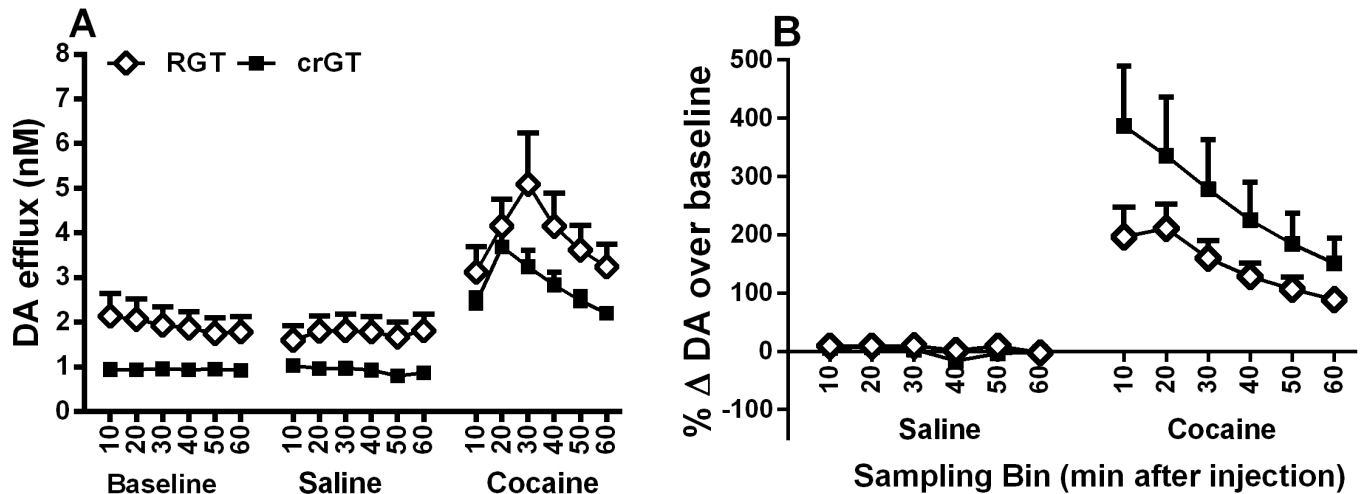


Figure 5-4 Results from microdialysis in the NAc after task experience.

(A) Raw NAc DA dialysate. Compared to rGT trained rats, crGT experienced animals showed significantly less basal efflux at baseline and after saline exposure, but comparable levels after cocaine administration. (B) Change in DA efflux over baseline. All animals exhibited significant potentiation in DA release after cocaine exposure, but this effect was slightly greater in crGT trained rats, indicating although these animals have less NAc DA at baseline, they may be slightly more reactive to cocaine. Values are averages of both left and right hemispheres \pm SEM.

Chapter 6: Investigating the influence of blunted nucleus accumbens dopamine on the expression of risky choice

6.1 Introduction

Addiction is a chronic relapsing disorder that afflicts millions of individuals worldwide (UNODC, 2015). Poor cost/benefit decision making is thought to mitigate addiction vulnerability by biasing choice towards high risk, high reward options despite future consequences (Bechara, 2003). Indeed, impaired choice as measured by the Iowa Gambling Task (IGT, a validated assay of real-world decision making) has been noted in a variety of addicted populations (Bechara and Damasio, 2002; Bolla et al., 2005; Verdejo-Garcia et al., 2007; Wang et al., 2012). Using a rodent analogue of the IGT, the rat gambling task (rGT), in which animals choose among four options that either have improbable but large rewards (risky) or likely small wins (optimal), we have shown risky choice is associated with increased drug seeking, relapse risk, and is worsened by cocaine self-administration (Ferland and Winstanley, 2016). Interestingly, adding win-paired audiovisual cues to the rGT (the cued rat gambling task, crGT) increased risky choice, was associated with greater cocaine self-administration, and made animals more susceptible to drug-induced decision-making deficits irrespective of baseline choice profile (chapter 4).

Previous studies have suggested repeated exposure to cues predictive of probabilistic outcomes, and repeatedly responding for uncertain rewards, can sensitize the dopamine (DA) system, in turn making the individual more sensitive to psychostimulants (Singer et al., 2012; Zack et al., 2014). Phasic DA release in the NAc has also been found after psychostimulant

administration (Willuhn et al., 2012) and is recruited during decision making in a risk-preferring manner, such that risk-prone animals exhibit greater DA release during presentation of a cue predictive of the uncertain option, whereas risk-averse animals show the opposite pattern option (Sugam et al., 2012). However, crGT trained rats were found to have less DA efflux within the NAc, suggesting a hypoactive mesostriatal DA network may subserve both sensitivity to cocaine and risky choice (chapter 5). Exploring whether dampened DA within the NAc affects decision making would provide significant insight as to how suboptimal choice and addiction susceptibility might transect at the neurobiological level.

Human and preclinical data depict clear but distinct roles for DA in these behavioural phenomena. In drug seeking, DA has been found to mediate acquisition of self-administration (Caine et al., 1999) and cue and context specific relapse (Willuhn et al., 2010). In terms of addiction vulnerability, the reward-deficiency hypothesis of addiction posits blunted DA system activity, either the result of chronic drug use (Volkow et al., 2009), or in individuals that naturally exhibit blunted DA activity (Blum et al., 2011a), fosters a greater sensitivity to the reward effects of drugs and cues associated with them (Blum et al., 2011b; Blum et al., 2012a). Animal data support this claim, as low doses of dopamine antagonists potentiate cocaine consumption (Caine and Koob, 1994b), whereas DAD₁ receptor knockout mice fail to acquire self-administration (Caine and Koob, 1994a), indicating blunted but not abolished DA activity mitigates the reinforcing effects of cocaine.

In decision making, DA release appears to have a much more complex function. In humans, reduction of tyrosine (a key substrate for the synthesis of DA) by administration of a branched-chained amino acids cocktail impaired decision making on the IGT (Sevy et al., 2006). On the rGT, upregulation of DA release by acute amphetamine administration increased risk-

aversion, whereas a D2-receptor antagonist improved decision making, suggesting both efflux and receptor activity have discrete control over biasing choice (Zeeb et al., 2009). In contrast, amphetamine administration had limited effects on crGT performance, whereas modulating D₃ receptor activity mediated risky choice (Barrus and Winstanley, 2016). However, these studies investigated the influence of acute, systemic manipulations of DA on decision making, but the reduced NAc DA observed in crGT rats may be the product of chronically downregulated DA after task experience. Likewise, blunted DA activity is more closely associated with prolonged drug use (Volkow et al., 2009), pointing to a relationship between prolonged compensatory changes in the DA system and addiction vulnerability. Whether acutely or chronically lowered NAc DA release influences risky choice has yet to be explored.

Although well-validated and reliable, pharmacological methods are limited by lack of cell specificity, pharmacokinetic properties of drugs administered, and can be damaging when targeting specific subregions via intracerebral microinfusions. With the advent of designer receptors exclusively activated by designer drugs (DREADDs), we are now able to selectively and reversibly downregulate cell activity using systemic drug challenges. When used in combination with a transgenic rat system, we can go a step further and target distinct cell types (i.e. DA expressing cells), to acutely or chronically modulate neurotransmitter activity. In this study, we used the inhibitory DREADD hM4D(Gi) in TH:Cre expressing rats to acutely and chronically inhibit DA release within the NAc in rGT and crGT trained rats via systemic clozapine-n-oxide (CNO) administration. After chronic inhibition, rats underwent a concomitant amphetamine challenge to determine how reduced DA release would impact the effect a potent psychostimulant had on gambling task performance. We hypothesized that chronically reducing

DA efflux would significantly impair decision making, and that this deficit would be exacerbated after amphetamine administration.

6.2 Additional Methods

Subjects

Thirty-two male long-evans rats were obtained from an in-house breeding program for transgenic animals that express cre-recombinase (Cre) in neurons that contain tyrosine hydroxylase (n=16 per transgene, TH:Cre^{+/-}, TH:Cre^{-/-}; TH:Cre rats from Rat Resource and Research Centre, RRRC, Columbia, MO; Long-Evans-Tg(TH-Cre)3.1Deis, RRRC #00659; wildtype rats obtained from Charles River, St. Constant, Canada; (Witten et al., 2011)). Offspring genotypes were determined using PCR as described previously (see general methods). Rats were weaned at post-natal day 21, housed two to three animals per cage, and had access to *ad libitum* standard rat chow and water until an average weight of approximately 300 g was reached. Animals were then transferred to the main vivarium, food-restricted to 85% of their free-feeding weight, and maintained in colony conditions previously described (see general methods).

Surgeries

Prior to task training, animals underwent aseptic stereotaxic surgery. Rats were anesthetised with isoflurane (5% induction, 2-3% maintenance), and the surgical site was cleaned and administered bupivacaine as a local anesthetic. Rats were bilaterally injected with Cre-dependent inhibitory DREADD AAV5-hSyn-DIO-hM4D(Gi)-mCherry (UNC Vector Core,

Durham, USA; titer 5.5×10^{12} or 4.6×10^{12} gc/ml; (Krashes et al., 2011)) into the NAc on the boundary between the shell and core (in keeping with previous methods (chapter 5); relative to bregma: anterior/posterior +1.7, medial/lateral ± 1.1 , and dorsal/ventral - 7.5; flat skull maintained at -3.3; (Paxinos and Watson, 1998)). Using 32 G stainless steel injectors (Plastics One, Roanoke, VA), PE tubing (Instech), and 10 ul syringes (Hamilton), 1 ul of AAV was infused at a rate of 0.1 ul/minute, and injectors left in place for 10 minutes to ensure solution fully diffused from the injector tip. Animals received ketapofen for pain management and recovered for a minimum of four days before behavioural training.

Drugs

Clozapine-n-oxide (CNO; Toronto Research Chemicals, Toronto, Canada) and d-amphetamine sulfate (Sigma Aldrich) were delivered via the intraperitoneal (i.p.) route in a volume of 1 ml/kg. Doses were calculated as the salt and dissolved in vehicle prior to injections. Amphetamine was dissolved in 0.9% sterile saline, and CNO was dissolved in 6% DMSO and 0.9% sterile saline.

CNO administration

Acute CNO administration began 11 weeks after viral infusions to allow adequate time for DREADD expression and behavioural stability. Using a diagram-balanced Latin square design to control for order effects (Zeeb et al., 2009; Cardinal and Aitken, 2013), the animals received intraperitoneal (i.p.) injections of vehicle (6% DMSO in saline), 0.3 mg/kg, 1.0 mg/kg, or 3.0 mg/kg CNO 30-min before the start of the behavioural session. Solutions were prepared fresh daily.

After acute administration and washout period, animals received i.p. injections of 1.0 mg/kg CNO twice per day for four weeks. The first injection occurred 30-min prior to the task session in the morning (between 10:30 am-1 pm) and the second injection was administered 6-7 hours later. CNO dissolved in DMSO was prepared on a weekly basis, aliquoted into individual tubes, and frozen at -20° C. Each day an aliquot was removed, thawed to room temperature, and diluted with saline.

Acute amphetamine challenge

After two weeks of bi-daily CNO administration, the animals received concomitant i.p. injections of saline vehicle, 0.3 mg/kg, and 1.0 mg/kg amphetamine in a Latin square design. CNO and amphetamine injections were given 30- and 10-min before the task, respectively.

Immunohistochemistry

At the end of the chronic dosing period, rats were sacrificed by transcardial perfusion of ice cold 10% phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Rats were injected with 120 mg/kg ketamine and 15 mg/kg xylazine i.p., perfused, and brains were extracted and stored in 4% paraformaldehyde for four to five days before being transferred to 30% sucrose solution for at least 72 hours. The brains were then frozen and sliced at 35 µm on a cryostat. Sections of the NAc and VTA were taken and stored as free-floating slices in PBS.

Sections were processed for mCherry and TH immunoreactivity (for antibodies see table 6.1). Tissue was washed in PBS (3 x 5 min) and incubated at room temperature for 1 hour in a PBS blocking solution containing 0.3% triton X and 5% normal goat serum. Sections were then incubated overnight at 4° C in anti-mCherry or anti-TH primary antibodies, washed for 2 x 5 min

in PBS, and incubated again overnight with opposite primary antibody. Sections were washed with PBS 3 x 5 min followed by a 2 hr incubation period with secondary Alexa 633 (mCherry) or Alexa 488 (TH) antibodies at room temperature. Sections were washed 3 x 5 min in PBS, and incubated in the subsequent secondary antibody for 2 hr. Tissue was washed 3 x 5 min with PBS, mounted onto gelatin-coated glass slides, and cover-slipped with VectaShield anti-fade Mounting Medium (Vector Laboratories, Burlingame, CA, USA). Expression of mCherry and TH was confirmed within the VTA and NAc using an AxioZoom V16 microscope (Zeiss, Germany).

Statistical Analyses

Behavioural data were analysed as described previously (see general methods). For acute drug challenges, dose was included as a within-subjects factor (CNO- four levels, vehicle, 0.3, 1, 3 mg/kg). Due to exclusion of animals that completed < 20 trials after amphetamine dosing, data for percent choice, score, and premature responding were analysed for each dose using an ANOVA with dose as a within-subjects variable (two levels: saline vs 0.3 mg/kg or 1 mg/kg). All remaining variables included the dose response curve as a within-subjects factor (three levels: saline, 0.3 mg/kg, 1 mg/kg). For the first two weeks of chronic CNO dosing, session (four levels: baseline, Day 1, Day 7, Day 14) was included as a within-subjects variable to determine whether CNO exposure affected task performance relative to baseline. Transgene status and task (two levels: task- crGT, rGT; transgene- Tg+ (TH:Cre^{+/+}), Tg- (TH:Cre^{-/-})) were between-subjects factors for all analyses.

6.3 Results

Histology

Histological verification of hM4D(Gi) expression resulted in six animals being excluded due to unilateral expression of the DREADD. Although the NAc shell/core boundary was targeted, most samples showed greater expression within the shell, likely due to richer VTA→NAc shell afferents at this coordinate. See figures 6.1 and 6.2 for expression.

Baseline Performance

Similar to previous experiments, rats exhibited significant choice preferences (table 6.2, Figure 6.3; choice $F_{2,45} = 18.829$, $p < 0.001$ with an overall preference for P2 (P1 vs P2- $t(25) = -6.695$, $p < 0.001$; P2 vs P3- $t(25) = 3.521$, $p = 0.002$; P2 vs P4- $t(25) = 4.731$, $p < 0.001$; P1 vs P3- $t(25) = -1.981$, $p = 0.059$; P1 and P3 vs P4, $t < -1.013$, $p > 0.321$). Visual inspection of the data suggests crGT rats had greater choice of P3 and less choice of P2, although due to individual variability, differences in choice did not reach statistical significance by task (choice x task- $F_{2,45} = 0.241$, $p = 0.868$). TH:Cre^{+/-} rats showed slightly longer collection latencies (Tg- $F_{1,22} = 3.551$, $p = 0.073$), albeit on the millisecond scale. There were no other significant differences in for any other rGT or crGT measure across sessions, by task, or by transgene ($F > 2.650$, $p = 0.112$).

Acute CNO Challenge

Acute downregulation of dopamine significantly improved decision making (figure 6.4A-B; dose x transgene- $F_{3,66} = 2.747$, $p = 0.050$; choice- $F_{2,51} = 22.003$, $p < 0.001$; dose x choice-

$F_{4,95} = 2.574$, $p = 0.038$) and post-hoc analyses revealed only TH:Cre^{+/-} animals showed significant changes in choice (Tg+: dose- $F_{3,27} = 2.935$, $p = 0.051$; choice: $F_{9,18} = 10.892$, $p = 0.001$; dose x choice- $F_{9,81} = 2.074$, $p = 0.041$; Tg- choice $F_{2,39} = 11.308$, $p < 0.001$; all remaining $F < 1.464$, $p > 0.243$) by choosing P3 significantly less with CNO on board (veh vs. 0.3 mg/kg- $t(10) = 2.362$, $p = 0.040$; vs. 1 mg/kg- $t(10) = 2.455$, $p = 0.034$; vs. 3 mg/kg- $t(10) = 2.544$, $p = 0.029$; $t < 1.852$, $p > 0.094$). CNO administration did not affect performance of any other task variable (table 6.3; $F < 2.313$, $p > 0.143$), indicating acute downregulation of NAc DA uniquely attenuated risky choice in DREADD-expressing rats.

Chronic CNO Administration

Two weeks of chronic CNO administration also significantly improved decision making (figure 6.5A-B; choice- $F_{2,41} = 17.693$, $p < 0.001$; session x choice x Tg- $F_{6,129} = 3.136$, $p = 0.007$). In TH:Cre^{+/-} rats, decision making showed marked changes across the two week period, trending towards different choice of individual options, most visibly increased choice of P2 (choice- $F_{7,16} = 7.073$, $p = 0.001$; session- $F_{3,27} = 4.342$, $p = 0.013$, session x choice- $F_{9,81} = 1.885$, $p = 0.066$). CNO administration did not alter TH:Cre^{-/-} rats choice preferences (choice- $F_{2,25} = 11.746$, $p = 0.0003$; all remaining $F < 2.185$, $p > 0.093$). Task experience accounted for changes in decision making (figure 6.5C-F; session x task- $F_{3,66} = 2.803$, $p = 0.047$) and post-hoc analyses revealed trend changes in decision making by task (session x task- $F_{3,27} = 2.651$, $p = 0.07$), albeit not for a specific option (choice x task, session x choice x task- $F < 1.292$, $p > 0.254$). Visibly rGT rats decreased choice of P3 and P4, whereas crGT rats mostly decreased choice of P4, suggesting the lack of cues facilitated shifts towards optimal decision making in the

absence of NAc DA. Surprisingly, improvements in choice were not reflected by a significant improvement in score (table 6.4; $F < 1.618$, $p > 0.194$).

Premature responding varied during the chronic CNO period (table 6.4; session x Tg- $F_{3,66} = 3.664$, $p = 0.017$) particularly in TH:Cre^{-/-} rats (Tg-: session- $F_{3,39} = 3.366$, $p = 0.028$; Tg+: $F < 2.011$, $p > 0.136$). Follow-up analyses revealed impulsive responding decreased from Day 1 to Day 7 ($t(14) = 2.565$, $p = 0.022$) and Day 14 ($t(14) = 2.262$, $p = 0.04$), which may be the result of animals habituating to the dosing regimen. TH:Cre^{+/-} and rGT trained animals showed slightly longer reward collection latencies during chronic dosing (transgene- $F_{1,22} = 3.510$, $p = 0.074$; task- $F_{1,22} = 3.638$, $p = 0.070$), although latencies did not potentiate across the epoch (session, session x task, session x Tg- $F < 1.805$, $p > 0.155$). Chronic CNO left remaining task measures unchanged (table 6.4; $F < 2.837$, $p > 0.106$).

Chronic CNO + Amphetamine Challenge

After two weeks of chronic CNO, rats underwent an acute, concomitant amphetamine challenge in which CNO and AMPH were administered prior to task performance. A low dose of amphetamine significantly affected decision making (Figure 6.6A-D; Dose- $F_{1,19} = 5.411$, $p = 0.031$; Dose x task x Tg- $F_{1,19} = 4.746$, $p = 0.042$; Choice $F_{2,41} = 18.121$, $p < 0.001$) and most prominently in crGT Tg+ rats (Dose x choice x task x Tg- $F_{3,57} = 4.537$, $p = 0.006$). Subsequent analyses of each task and transgene found although rGT TH:Cre^{+/-} rats' choice changed after 0.3 mg/kg of amphetamine, this was not reflected by increased selection for another choice, but instead reduction in preference for a single option (rGT, Tg+: dose- $F_{1,3} = 8.489$, $p = 0.062$; choice- $F_{3,9} = 1.982$, $p = 0.187$; Tg-: choice- $F_{1,9} = 6.939$, $p = 0.018$). In contrast, crGT TH:Cre^{+/-} rats showed significant changes in choice, and trended towards increased optimal choice (Tg+:

dose- choice- $F_{3,12} = 14.056$, $p < 0.001$; dose x choice- $F_{3,11} = 0.073$; Tg-: Choice- $F_{3,18} = 4.934$, $p = 0.01$). Although not significant at the individual choice level, crGT TH:Cre^{+/-} exhibited a significant improvement in score (Figure 6.7B; crGT Tg+; dose x task- $F_{1,19} = 9.389$, $p = 0.006$; crGT- dose- $F_{1,10} = 10.764$, $p = 0.008$; dose x Tg- $F_{1,10} = 4.752$, $p = 0.054$; crGT Tg+: dose- $F_{1,4} = 11.628$, $p = 0.027$), an effect not seen in rGT or crGT Tg- rats (Figure 6.7A, C, F; $F < 2.839$, 0.108). These data suggest that not only does chronic downregulation of DA optimize decision making, but potentiation of DA facilitates this shift in choice in crGT rats.

In contrast, 1 mg/kg of amphetamine impaired decision making regardless of task experience or transgene status by significantly increasing choice of P1 and P4 while decreasing choice of P2 (Figure 6.6 A-D; dose- $F_{1,14} = 29.551$, $p < 0.001$; Choice $F_{3,36} = 7.537$, $p = 0.001$; dose x choice- $F_{3,41} = 4.739$, $p = 0.007$; paired-sample t-tests: P1- $t(17) = -5.175$, $p < 0.001$; P2- $t(17) = 2.235$, $p = 0.039$; P3- $t(17) = 0.016$, $p = 0.988$; P4- $t(17) = 0.039$). Score was not significantly changed at this dose ($F < 2.434$, $p > 0.141$). These data indicate blunted DA does not mitigate decision-making deficits brought on by a higher amphetamine dose, indicating the improvements in choice seen at the lower dose are likely the result of a balance between reduced NAc DA and upregulated efflux in other brain structures.

In all rats, both doses of amphetamine exacerbated motor impulsivity as reflected by significant increases in premature responses (Figure 6.8; 0.3 mg/kg amphetamine: dose- $F_{1,21} = 30.928$, $p < 0.001$; 1 mg/kg- dose- $F_{1,18} = 82.651$, $p < 0.001$). Amphetamine also universally and dose dependently reduced the number of trials completed (Table 6.5; dose- $F_{2,36} = 41.453$, $p < 0.001$; paired-sample t-tests: Sal vs 0.3 mg/kg - $t(24) = 4.301$, $p < 0.001$; vs 1 mg/kg- $t(24) = 7.182$, $p < 0.001$). Latencies to make a choice were also significantly reduced in all rats (Table 6.5; dose- $F_{2,42} = 9.280$, $p < 0.001$), however TH:Cre^{+/-} rats exhibited faster latencies at both

doses (dose x Tg- $F_{2,42} = 4.217$, $p = 0.021$; Tg+ only: dose- $F_{1,26} = 12.450$, $p = 0.004$; Sal v 0,3-
t(9) 4.359, $p = 0.002$; Sal v 1- t(9) = 2.992, 0.015) whereas Tg- rats had significant reduced
choice latencies after 1 mg/kg of amphetamine (dose- $F_{2,26} = 5.163$, $p = 0.013$; Sal vs 0.3 – t(14)
= 0.534, $p = 0.602$; Sal v 1 mg/kg- t(14) = 3.466, $p = 0.004$), suggesting reduced DA tone may
expedite choice during the anticipation period. The number of omitted responses or latency to
collect reward were unaffected by amphetamine administration, and there were no other
significant interactions of dose, transgene, or task for all measures ($F > 2.699$, $p < 0.115$).

6.4 Discussion

Here we show that modulating DA efflux within the NAc influences preference for uncertain outcomes. TH:Cre^{+/-} rats expressing inhibitory DREADD hM4D(Gi) in dopaminergic projections to the NAc showed improved decision making after acute CNO administration, an effect somewhat maintained throughout chronic CNO exposure. In contrast, CNO administration did not affect choice patterns in transgene negative control animals. Interestingly, TH:Cre^{+/-} crGT rats exhibited further improved choice after a concomitant amphetamine challenge during the chronic CNO period, indicating a complex interaction between reductions in DA release within the NAc, the response to a psychostimulant, and the presence of reward-paired cues.

We previously observed that crGT experience was associated with less NAc DA compared to rGT rats and increased risky decision making, leading to our hypothesis that reducing NAc DA may increase risky choice. However, the current data support the opposite: acute CNO reduced uncertain choice in TH:Cre^{+/-} rats regardless of task experience, suggesting greater efflux maintains risky choice in general. Indeed, previous evidence has shown DA is important for modulating biases in decision making under uncertainty. In a rodent betting task,

systemic amphetamine administration potentiated choice of uncertain reward in risk-averse animals (Cocker et al., 2012), and repeated exposure to amphetamine increased uncertain choice on a probabilistic discounting task (Floresco and Whelan, 2009). These data collectively suggest DA tone is imperative for the formulation of risk-prone biases, regardless of cue exposure.

Chronic CNO improved decision making in rats expressing the DREADD regardless of task, although it produced only approached significance in choice selection. However, visibly TH:Cre^{+/-} rats show reductions in choice of the risky options and increased choice of P2. The lack of significance seen here may be due to the stress of a rigorous injection schedule, resulting in choice variability. Interestingly, changes in decision making during chronic CNO exposure varied depending on task experience, as rGT rats showed decreases in selection of P3 and P4, whereas crGT rats showed only reductions in P4. These data indicate even with blunted NAc DA, cues may make the animal slightly more resistant to changes in risky choice. Replication of this experiment would provide the necessary power to address both the lack of significant change in choice and differences in task performance.

Data from both acute and chronic CNO challenges indicate NAc DA contributes to a predisposition for risky choice, but does not drive disadvantageous decision making, *per se*. It is likely the lower accumbal DA levels seen in crGT rats are the product of a compensatory response to task-induced DA release, which may be accompanied by other reflexive receptor changes which contribute to the expression of risky choice and sensitivity to drugs of abuse (as seen in chapter four). Given previous acute DA receptor challenge data, several pieces of evidence indicate modulation of D₂-like receptors facilitates risky decision making. Indeed, inhibiting the D₂ receptor improved rGT performance (Zeeb et al., 2009) and manipulating D₃ receptor activity mitigated crGT risky choice (Barrus and Winstanley, 2016). Additional data

from the field implicate the D₂ family in various aspects of decision making, including cognitive flexibility (Groman et al., 2016), valuation of reward (Caine and Koob, 1994b), and motivation (Linnet et al., 2011; Trifilieff et al., 2013; Cocker et al., 2017). Looking to the exacerbated decision-making deficits seen in chapters three and four, cocaine may induce neuroadaptations which alter D₂/D₃ receptor expression (Vorel et al., 2002; Volkow et al., 2006; Di Ciano, 2008; Volkow et al., 2010; Boileau et al., 2012), biasing choice in favour of risk. The DREADD expression profile in the NAc also implicates the shell subregion in particular, a nucleus previously implicated in drug addiction, and shown to be critical in mediating the behavioural influence of reward-predictive cues and changes in reward magnitude (Koob, 2003; Beyene et al., 2010; Stopper and Floresco, 2011; Sackett et al., 2017). Future studies investigating receptor expression within the NAc shell or manipulations of receptor activity in this region may shed light on the post-synaptic contribution to risk-preference.

Surprisingly, neither acute nor protracted downregulation of NAc DA affected premature responding, nor did it block amphetamine-induced impulsivity. This is unexpected, as studies have found increasing systemic DA tone via amphetamine or GBR12909 (a DA reuptake inhibitor) administration increases motor impulsivity on the rGT (Zeeb et al., 2009; Baarendse et al., 2013) and five-choice serial reaction time task (Navarra et al., 2008). Increasing DA efflux in the NAc has also been associated with behavioural disinhibition (Pattij et al., 2007; Economidou et al., 2012). Therefore, we anticipated CNO administration would reduce premature responding in general or blunt amphetamine-induced impulsivity. While amphetamine did increase premature responding in all animals, CNO administration did not mitigate this effect. The reasons for this may be two-fold. Firstly, evidence shows modulation of DA tone in the core, but not shell, increased premature responding (Economidou et al., 2012). Verification of viral

expression found that at this NAc coordinate, the VTA afferents were richer to the shell, whereas core expression was sparse. Therefore, cells transfected in this study may not affect impulsivity. Secondly, although TH:Cre rats uniquely express Cre in TH cells, on average 70-80% of TH cells will express Cre, resulting in some DA cells not expressing the DREADD (Witten et al., 2011). Therefore, it is possible previous pharmacological manipulations affected a greater number of DA cells innervating the NAc, leading to more robust modulation of premature responding compared to that mediated by DREADD-regulated expression in this study.

After a high dose of amphetamine, animals showed marked impairments in decision making, consistent with previous work (Zeeb et al., 2009; Barrus and Winstanley, 2016). From these data we can conclude amphetamine-induced choice deficits are not dependent on NAc DA release, but may be the result of both upregulated DA and other neurochemical systems like noradrenaline (NA; (Eiden and Weihe, 2011). Indeed, a previous study found that co-administration of a DA and NA reuptake inhibitor significantly increased choice of P1 and P3/P4 on the rGT, an effect not achieved by upregulated DA release alone (Baarendse et al., 2013). Also, the impairment in choice caused by higher doses of amphetamine on the rGT cannot be blocked by co-administration of a D₁ or D₂ receptor antagonist (Zeeb et al., 2013). It may therefore be unsurprising that down-regulation of NAc DA did not impact amphetamine's ability to shift preference towards P1 and P4 in the current study.

Interestingly, a low dose of amphetamine further improved decision making only in TH:Cre^{+/-} crGT rats. As such, downregulation of NAc DA release sensitised these rats' response to administration of the psychostimulant. The neurochemical basis of this effect is currently unclear, but may involve DA release in brain regions other than the NAc, or neurotransmitters other than DA (Baarendse et al., 2013). The observation that low, systemic amphetamine and

downregulation of NAc DA work synergistically to improve decision making suggests an inverted-U relationship between tonic/phasic DA release and choice. There are extant data which show dopaminergic agonists have an optimal dose to improve cognitive performance (Linssen et al., 2014; Turner and Burne, 2016), whereas hyperdopaminergic activation has been shown to exacerbate cognitive biases including perception of wins, choice under uncertainty, and risk-seeking (Winstanley et al., 2011; Cocker et al., 2012; Norbury et al., 2013). Previous data have shown NAc phasic DA is recruited based on choice preference (i.e. risk-prone animals exhibit greater DA release during cue presentation predictive of uncertain options; (Sugam et al., 2012). It is possible by abrogating VTA DA inputs, these choice biases may be rescinded, and DA activity in other brain regions, such as the prefrontal cortex (PFC), may serve to optimize choice. A recent study found simultaneous modulation of DA receptor activity within the medial PFC and contralateral NAc inactivation reduced uncertain choice on a probabilistic discounting task (Jenni et al., 2017). Manipulation of DA receptor activity in the PFC and inhibition of NAc glutamatergic inputs also reduced cue-induced reinstatement, suggesting this network may be particularly important for cue-mediated behaviours (McGlinchey et al., 2016). However, we did not test involvement of DA in other brain structures, and future studies investigating network dynamics are imperative to dissociate contributions of DA in different brain regions in decision-making biases.

Although amphetamine preferentially increases synaptic DA, this drug also acts upon the vesicular monoamine transporter, incidentally affecting release of other neurotransmitters including NA and serotonin (Eiden and Weihe, 2011). Therefore, the changes in choice seen after a low dose of amphetamine in crGT rats may be the result not of DA, but another monoamine. Previous work has shown increasing synaptic NA by atomoxetine administration

improved rGT performance (Baarendse et al., 2013), and modulation of the serotonin system by a 5HT_{1a} agonist increased risky choice on the rGT, whereas a 5HT_{2c} antagonist improved choice on the crGT (Zeeb et al., 2009; Adams et al., 2017). These neurotransmitters play a prominent role in choice, and may underlie the improvement in decision making in lieu of NAc DA release in crGT animals.

What remains unclear from the current data is whether chronic downregulation DA is necessary for amphetamine to improve choice in crGT rats, as we did not do a concurrent acute CNO/amphetamine challenge. The prospect of decision making being optimized under acute conditions is promising, and may offer an interesting research timepoint to explore for administration of potential therapeutics.

It is important to note that crGT rats, although visibly showed greater choice of P3 and P4, were statistically indistinguishable from rGT trained animals. It is possible surgery before task experience and expression of the virus disrupted NAc activity, leading to changes in task performance. However, this is unlikely, as animals did exhibit choice preferences, and we have found inhibition of NAc shell activity during crGT acquisition did not change decision making preferences at baseline (Barrus & Winstanley, in preparation). Exclusion of rats due to insufficient DREADDs expression may have increased variability within each task group, affecting significance detection. Furthermore, given previous results which found all crGT TH:Cre rats exhibited lowered NAc DA (chapter five), we believe the DA release in this cohort would be comparable. We did not confirm CNO depression of cell activity in TH:Cre^{+/-} rats, but instead relied upon differential behavioural expression between transgenic groups. Previous work has shown hM4D(Gi) activation reduced VTA spiking and cFos expression, and is associated with attenuated behaviour (Beloate et al., 2016). Importantly, changes in behaviour

here are robust and consistent, supporting the conclusion CNO uniquely affected DA cells in TH:Cre^{+/+} rats. However, replication of this study should incorporate a greater sample size and neurochemistry or electrophysiological assays to confirm CNO reduction in TH neuronal activity.

It is also imperative to consider a recent report which found that, despite being engineered to respond only to CNO, activation of the hM DREADDs is actually the result of clozapine binding, which becomes available after CNO is metabolized in the liver (Gomez et al., 2017). Therefore, to draw conclusions from DREADDs data, it is essential to 1) have a control group which does not express the DREADD, to be certain there is no spurious effect of CNO, and 2) determine whether behavioural data are the result of general clozapine binding in the central nervous system. To address the former, TH:Cre^{-/-} rats were used in this study, did not express the DREADD, and did not show any behavioural response to CNO. To explore the latter, we can compare the results here with previous reports using D₂ and 5HT_{2A} antagonists, the main sites of action for clozapine (Meltzer, 2002). Interestingly, chronic CNO exposure changed choice in favour of P2, similar to previous results on the rGT in which a D2 antagonist eticlopride optimized choice on the rGT (Zeeb et al., 2009), but did not impact crGT choice (Barrus and Winstanley, 2016). Provided that CNO improved choice regardless of task experience, it is likely the effect seen in this study is likely due to changes in efflux rather than general D₂ antagonism. Similarly, systemic 5-HT_{2A} antagonist M100907 had no impact on choice performance in the rGT or crGT, but significantly attenuated premature responses (Adams et al., 2017), an effect not seen with CNO administration here. Therefore, these data are likely the result of specific DREADDs activation. However, future studies should incorporate a

clozapine challenge to determine if systemic clozapine replicates behavioural effects seen with CNO.

In conclusion, these data show that NAc DA efflux helps to foster risk tolerance, and decision-making biases can be alleviated by reducing NAc DA release. The current results further our foundational knowledge of DA's involvement in the development of poor cognitive performance, and may provide insight into avenues for therapeutic development for psychiatric disorders in which risky decision making is a key cognitive deficit, including addiction.

6.5 Tables

mCherry	1:700 dilution chicken polyclonal, Abcam, ab205402	1:500 dilution goat polyclonal IgY Alexa Fluor® 633 conjugate, Thermo Fisher Scientific, cat. no. A-21103
Tyrosine hydroxylase	1:100 dilution rabbit polyclonal, Millipore, ab152	1:500 dilution goat polyclonal, Alexa Fluor® 488 conjugate, Thermo Fisher Scientific, cat. no. A-11034

Table 6-1 Antibodies used for immunohistochemistry.

<i>Behavioural Measure</i>	<i>Group</i>			
	<i>rGT TH:Cre^{+/-}</i>	<i>rGT TH:Cre^{-/-}</i>	<i>crGT TH:Cre^{+/-}</i>	<i>crGT TH:Cre^{-/-}</i>
Score	13.66 ± 40.59	49.65 ± 15.70	15.91 ± 14.47	-12.06 ± 21.72
Premature Responses	14.52 ± 4.77	17.46 ± 2.27	18.18 ± 2.40	15.05 ± 3.80
Trials Completed	100.81 ± 15.29	94.69 ± 7.11	85.02 ± 5.71	81.92 ± 10.21
Omissions	0.93 ± 0.49	1.81 ± 1.16	1.00 ± 0.40	0.75 ± 0.38
Choice Latency	1.27 ± 0.27	1.08 ± 0.19	1.16 ± 0.22	1.13 ± 0.11
Collection Latency	0.90 ± 0.20	0.73 ± 0.05	0.83 ± 0.13	0.58 ± 0.03

Table 6-2 Baseline rGT and crGT performance in TH:Cre^{+/-} and ^{-/-} rats prior to CNO administration.

Values are means from final three baseline sessions ± SEM.

		CNO Dose			
Group	Behavioural Measure	Veh	0.3 mg/kg	1 mg/kg	3 mg/kg
rGT TH:Cre ^{+/-}	Score	37.44 ± 36.94	50.67 ± 32.73	49.80 ± 36.48	45.25 ± 36.70
	Premature Responses	15.47 ± 8.09	18.90 ± 8.91	18.93 ± 8.65	13.55 ± 5.65
	Trials Completed	88.24 ± 23.21	93.82 ± 17.58	93.22 ± 15.64	106.42 ± 15.68
	Omissions	1.60 ± 1.17	2.20 ± 1.43	1.20 ± 0.73	2.40 ± 0.87
	Choice Latency	1.66 ± 0.51	1.47 ± 0.43	1.78 ± 0.73	1.65 ± 0.51
	Collection Latency	0.87 0.32	2.64 ± 1.82	0.97 ± 0.28	1.02 ± 0.28
rGT TH:Cre ^{-/-}	Score	59.56 ± 20.68	72.32 ± 17.59	69.81 ± 20.32	66.73 ± 21.69
	Premature Responses	18.01 ± 3.14	20.27 ± 4.73	18.50 ± 3.46	21.01 ± 3.90
	Trials Completed	98.76 ± 10.27	100.44 ± 11.81	99.01 ± 10.34	94.01 ± 9.53
	Omissions	1.57 ± 1.00	1.00 ± 0.85	0.86 ± 0.59	1.00 ± 0.72
	Choice Latency	1.24 ± 0.24	1.26 ± 0.19	1.31 ± 0.25	1.26 ± 0.22
	Collection Latency	0.83 ± 0.11	0.88 ± 0.14	0.89 ± 0.14	0.81 ± 0.10
crGT TH:Cre ^{+/-}	Score	36.05 ± 15.77	46.47 ± 14.72	45.42 ± 17.53	57.52 ± 14.06
	Premature Responses	12.73 ± 2.97	15.21 ± 4.42	15.67 ± 8.41	14.94 ± 7.41
	Trials Completed	97.18 ± 8.44	95.67 ± 9.67	86.52 ± 11.72	89.35 ± 7.18
	Omissions	1.00 ± 0.52	1.83 ± 0.48	1.50 ± 0.85	1.50 ± 0.62
	Choice Latency	1.34 ± 0.31	1.49 ± 0.42	1.59 ± 0.41	1.54 ± 0.29
	Collection Latency	0.90 ± 0.08	0.78 ± 0.09	0.79 ± 0.12	1.06 ± 0.27
crGT TH:Cre ^{-/-}	Score	10.76 ± 26.54	22.63 ± 26.81	25.29 ± 23.04	15.87 ± 26.02
	Premature Responses	19.19 ± 3.67	17.64 ± 4.53	19.14 ± 2.67	17.21 ± 2.92
	Trials Completed	77.28 ± 8.89	80.03 ± 11.46	78.54 ± 10.50	80.80 ± 10.70
	Omissions	0.38 ± 0.26	0.63 ± 0.50	0.50 ± 0.27	1.00 ± 0.63
	Choice Latency	1.32 ± 0.22	1.39 ± 0.27	1.26 ± 0.21	1.23 ± 0.16
	Collection Latency	0.63 0.07	0.61 0.08	0.62 0.06	0.63 0.08

Table 6-3 rGT and crGT performance after acute CNO administration in TH:Cre^{+/-} and ^{-/-} rats.

Values are averages per dose ± SEM.

Group	Behavioural Measure	Day of Administration		
		1 Day	7 Days	14 Days
rGT TH:Cre ^{+/-}	Score	34.07 ± 35.60	22.52 ± 14.42	33.98 ± 38.72
	Premature Responses	11.09 ± 6.07	12.64 ± 4.25	15.66 ± 6.31
	Trials Completed	109.20 ± 16.50	103.20 ± 14.94	109.80 ± 17.46
	Omissions	1.00 ± 0.45	2.40 ± 1.69	0.60 ± 0.40
	Choice Latency	1.37 ± 0.27	1.44 ± 0.33	1.45 ± 0.36
	Collection Latency	1.04 ± 0.23	0.92 ± 0.23	1.10 ± 0.31
rGT TH:Cre ^{-/-}	Score	46.08 ± 15.65	47.02 ± 16.06	41.22 ± 16.71
	Premature Responses	16.58 ± 4.62	11.17 ± 2.44	12.35 ± 2.80
	Trials Completed	93.71 ± 8.21	103.29 ± 9.22	100.46 ± 9.92
	Omissions	1.29 ± 0.57	2.00 ± 1.84	0.29 ± 0.18
	Choice Latency	1.26 ± 0.32	1.34 ± 0.33	1.46 ± 0.27
	Collection Latency	0.78 ± 0.10	0.76 ± 0.06	0.84 ± 0.07
crGT TH:Cre ^{+/-}	Score	15.37 10.97	21.81 24.87	25.36 12.04
	Premature Responses	11.50 ± 4.12	18.63 ± 6.24	13.95 ± 3.47
	Trials Completed	88.00 ± 5.76	86.18 ± 6.64	89.83 ± 5.62
	Omissions	1.17 ± 0.48	0.67 ± 0.67	0.67 ± 0.21
	Choice Latency	1.32 ± 0.27	1.17 ± 0.29	1.47 ± 0.42
	Collection Latency	0.78 ± 0.08	0.74 ± 0.11	0.78 ± 0.11
crGT TH:Cre ^{-/-}	Score	-7.84 ± 10.96	-9.68 ± 16.24	-11.50 ± 24.00
	Premature Responses	20.13 ± 5.32	14.94 ± 5.14	13.73 ± 4.40
	Trials Completed	82.01 ± 11.10	84.01 ± 10.88	81.39 ± 9.15
	Omissions	0.13 ± 0.12	0.25 ± 0.16	0.50 ± 0.38
	Choice Latency	0.88 ± 0.10	1.19 ± 0.16	1.13 ± 0.12
	Collection Latency	0.58 ± 0.05	0.58 ± 0.05	0.58 ± 0.05

Table 6-4 rGT and crGT performance during the first two weeks of CNO administration in TH:Cre^{+/-} and ^{-/-} rats.

Numbers in bold denote significance (p< 0.05) by session. Values are averages per timepoint ± SEM.

Group	Behavioural Measure	Amphetamine Dose		
		Saline	0.3 mg/kg	1 mg/kg
rGT TH:Cre ^{+/-}	Trials Completed	111.03 ± 18.96	90.04 ± 11.27	55.66 ± 12.57
	Omissions	1.25 ± 0.95	0.20 ± 0.20	0.80 ± 0.80
	Choice Latency	0.96 ± 0.16	0.65 ± 0.10	0.83 ± 0.11
	Collection Latency	0.90 ± 0.28	0.84 ± 0.20	0.71 ± 0.21
rGT TH:Cre ^{-/-}	Trials Completed	101.30 ± 7.36	90.87 ± 9.58	53.50 ± 9.39
	Omissions	1.43 ± 1.43	0.30 ± 0.18	0.14 ± 0.14
	Choice Latency	1.06 ± 0.34	1.07 ± 0.43	0.75 ± 0.18
	Collection Latency	0.82 ± 0.05	0.66 ± 0.06	1.02 ± 0.50
crGT TH:Cre ^{+/-}	Trials Completed	90.67 ± 6.69	73.75 ± 10.73	47.08 ± 10.20
	Omissions	1.17 ± 0.60	0.33 ± 0.21	0 ± 0
	Choice Latency	1.40 ± 0.25	0.66 ± 0.09	0.82 ± 0.13
	Collection Latency	0.73 ± 0.09	0.58 ± 0.09	0.52 ± 0.04
crGT TH:Cre ^{-/-}	Trials Completed	85.26 ± 11.23	75.65 ± 10.74	52.58 ± 6.81
	Omissions	0.37 ± 0.26	0.37 ± 0.37	0.12 ± 0.12
	Choice Latency	1.12 ± 0.16	0.97 ± 0.22	0.57 ± 0.09
	Collection Latency	0.57 ± 0.03	0.52 ± 0.04	0.45 ± 0.03

Table 6-5 rGT and crGT performance during concomitant amphetamine challenge and CNO administration in TH:Cre^{+/-} and ^{-/-} rats.

Numbers in bold denote significance (p< 0.05) by dose. Values are averages per dose ± SEM.

6.6 Figures

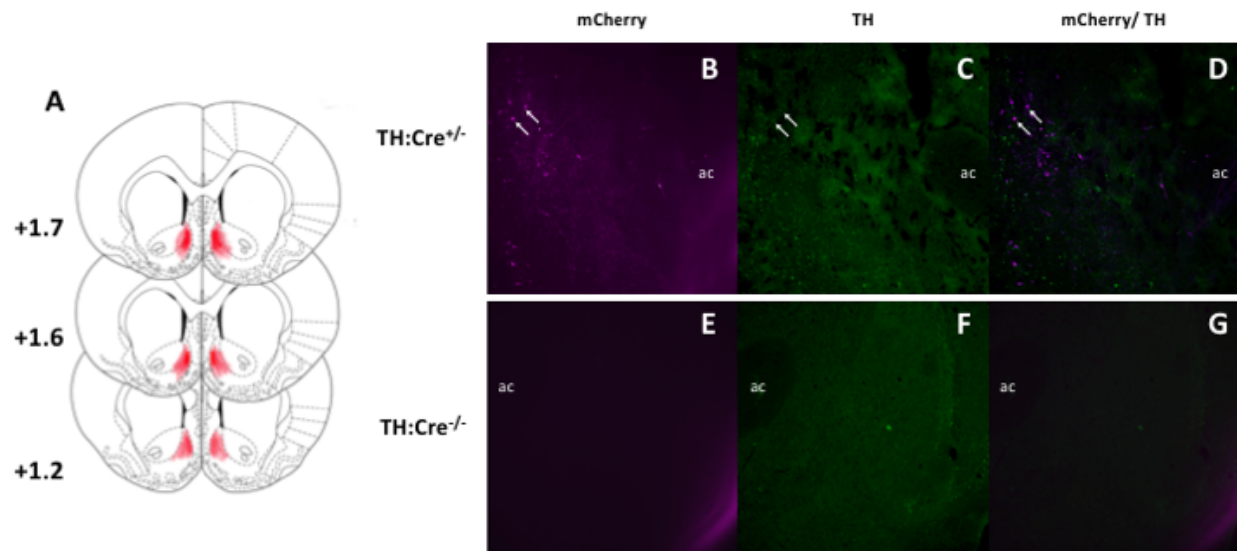


Figure 6-1 Confirmed viral expression within the NAc in TH:Cre^{+/-} and TH:Cre^{-/-} rats.

A) Depicts extent of expression in NAc DA terminals at specific anterior-posterior coordinates from bregma. Darker red colour indicates concentrated expression, whereas lighter pink depicts small clusters or singular cell expression. Most TH:Cre^{+/-} rats showed the greatest expression in medial NAc (reproduced and modified from (Paxinos and Watson, 1998)). B-D) Representative example of concentrated terminals expressing mCherry in the TH:Cre^{+/-} NAc in the left hemisphere relative to the anterior commissure (ac). E-G) Representative right hemisphere of TH:Cre^{-/-} NAc terminals. Note normal expression of TH inputs, but no expression of mCherry.

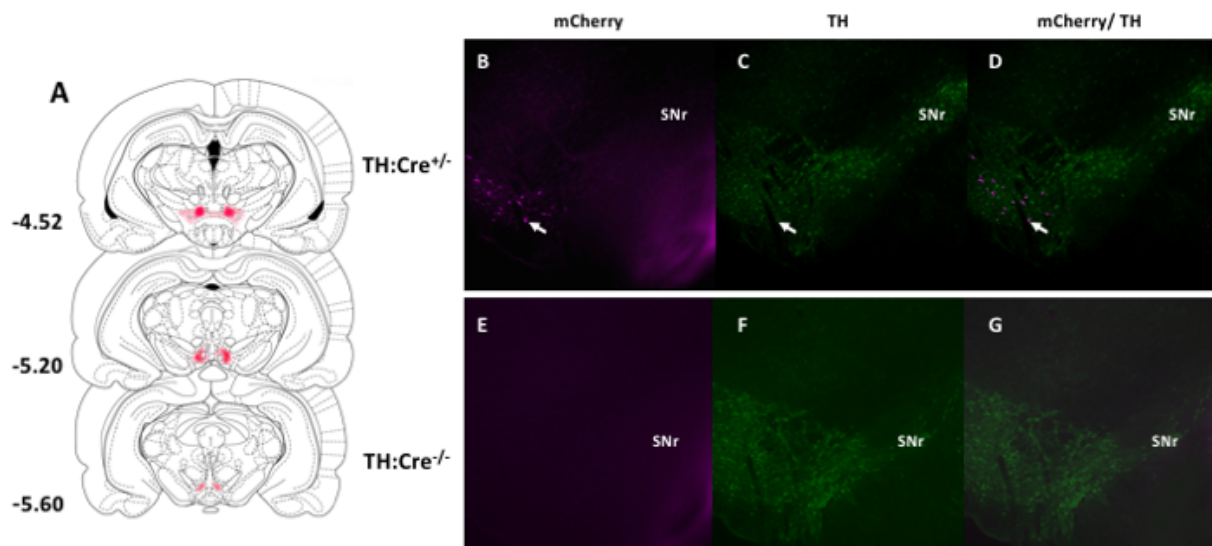


Figure 6-2 Confirmed viral expression within the VTA in TH:Cre^{+/−} and TH:Cre^{−/−} rats.

- A) Depicts extent of expression in VTA cell bodies at specific anterior-posterior coordinates relative to bregma. Darker red colour indicates concentrated expression, whereas lighter pink depicts small clusters or singular cell expression. Most TH:Cre^{+/−} rats showed the greatest expression in the most anteromedial aspect of the VTA (reproduced and modified from (Paxinos and Watson, 1998). B-D) Representative example of concentrated cell bodies expressing mCherry in the TH:Cre^{+/−} VTA as opposed to adjacent TH⁺ substantia nigra (SNr) nuclei. E-G) TH:Cre^{−/−} VTA neurons show normal expression of TH but no mCherry. All micrographs are of the left hemisphere.

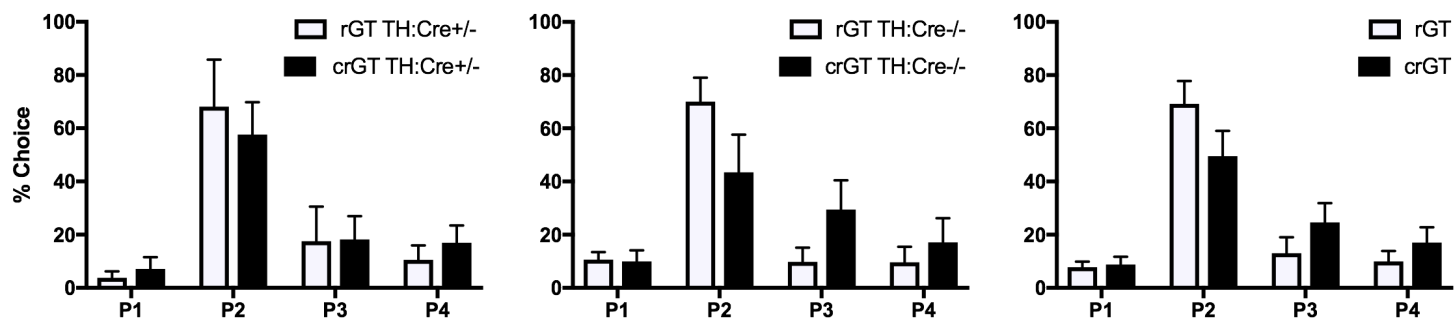


Figure 6-3 Choice preference at baseline in rGT and crGT TH:cre^{+/-} and ^{-/-} rats.

Values presented are averages \pm SEM.

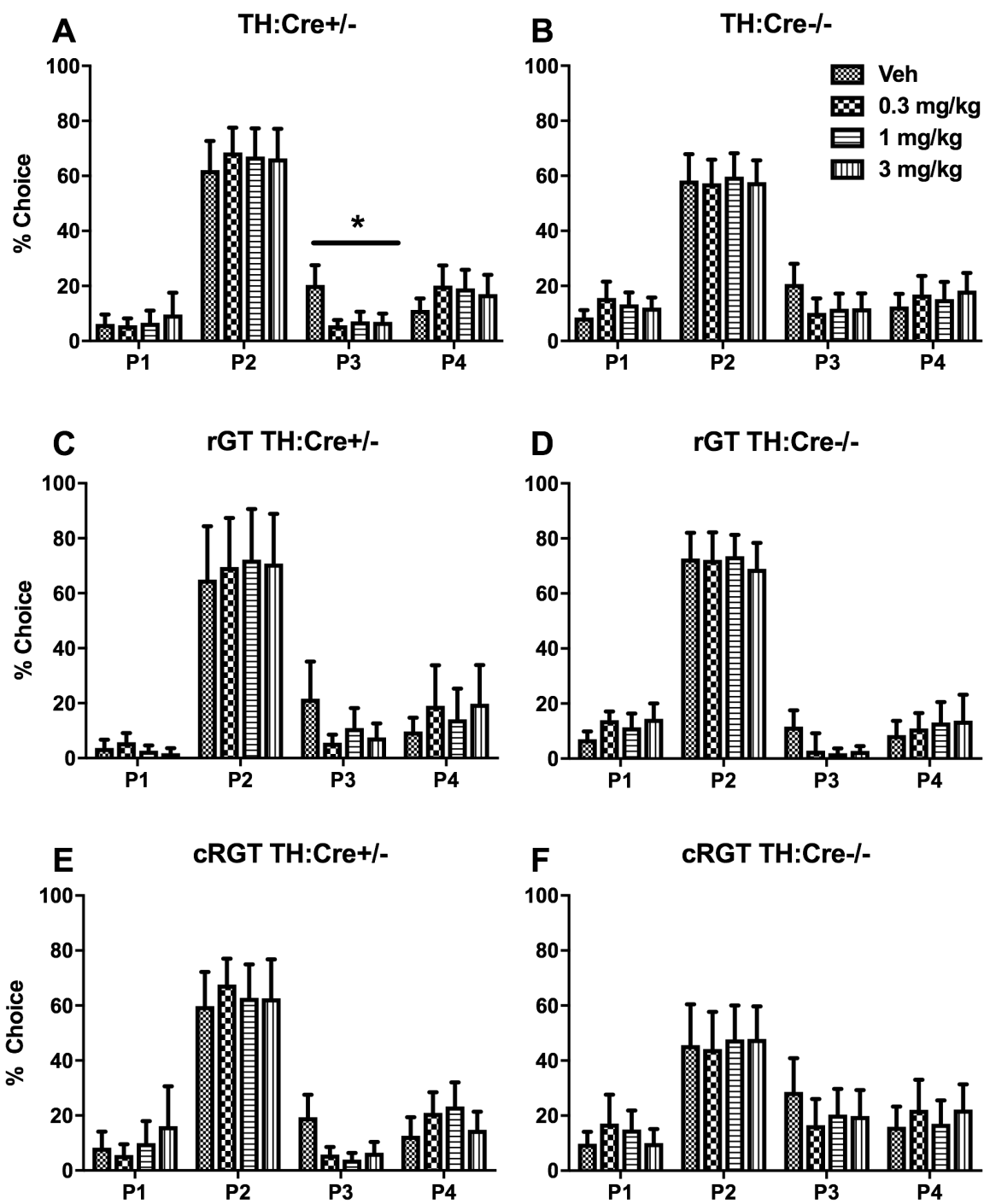


Figure 6-4 Decision making performance after acute CNO administration.

A, C, E) All three doses of CNO significantly reduced choice of P3 in TH:Cre^{+/-} regardless of task experience, whereas TH:Cre^{-/-} animals' choice remained unchanged (panels B,D,F). Asterisk denotes $p < 0.05$. Values presented are averages \pm SEM.

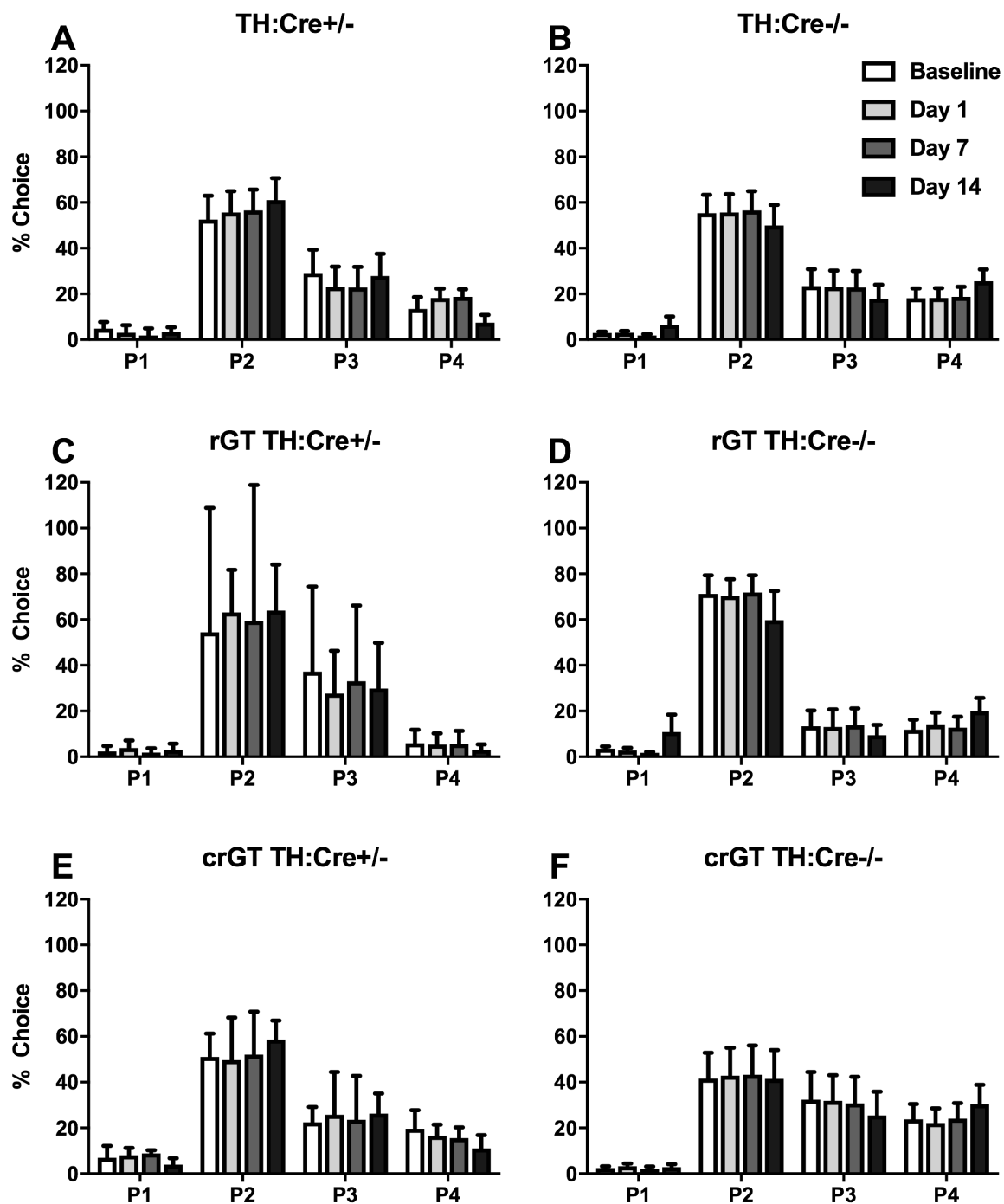


Figure 6-5 Individual choice preference by transgene group at baseline and after 2 weeks of chronic CNO administration.

A, C, E) Rats expressing hM4D(Gi) exhibited significant changes in choice, visibly in favour of P2. B, D, F)

Decision making was unaffected in TH:Cre^{-/-} counterparts. Values are averages \pm SEM.

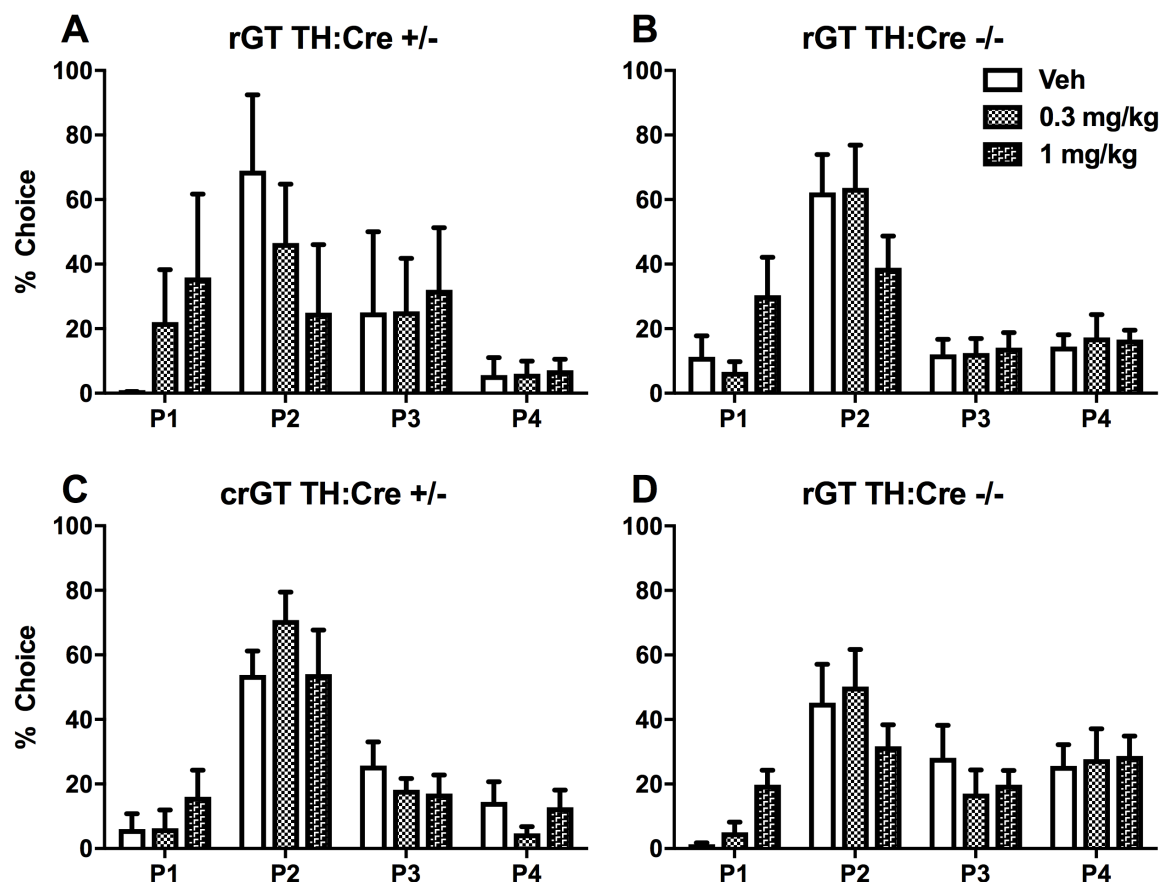


Figure 6-6 Individual choice preference by transgene group at after concomitant CNO + amphetamine challenge.

A) At the low dose of amphetamine, rGT TH:Cre^{+/-} animals showed changes in decision making, reducing preference for a single option. C) In contrast, crGT TH:Cre^{+/-} rats showed marked, albeit trend, improvements in decision making. A-D) Although TH:Cre^{-/-} rats did not show changes in choice after 0.3 mg/kg amphetamine, all animals showed significant impairments after 1 mg/kg of drug, reducing choice of P2 in favour of P1 and P4. Values are averages \pm SEM.

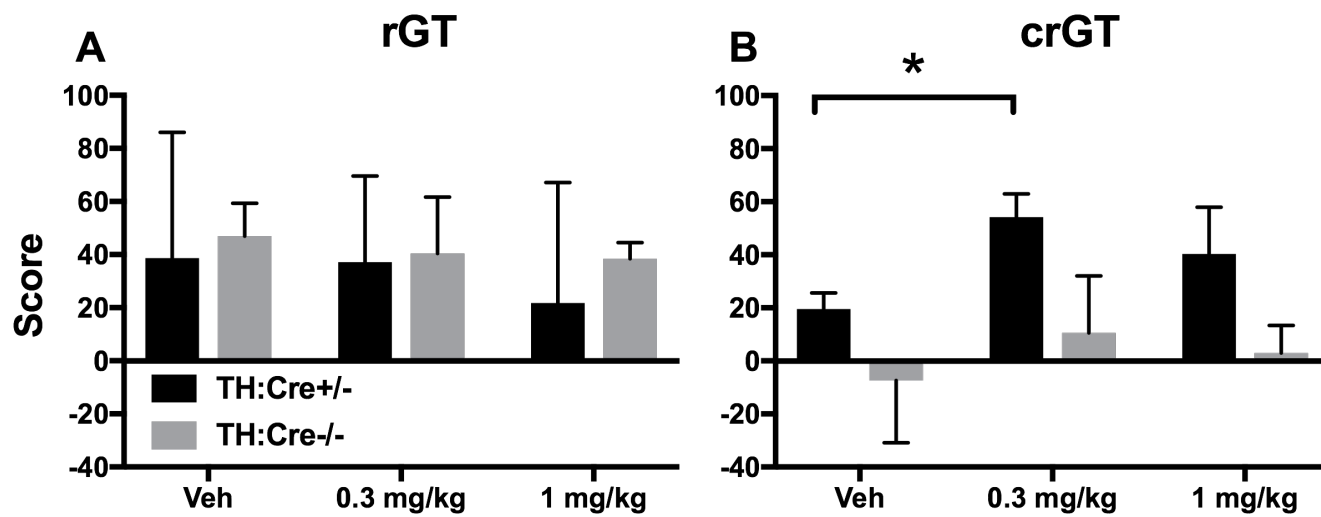


Figure 6-7 Choice as measured by the score variable after amphetamine administration.

A) Score was unchanged in both rGT transgene groups. B) In contrast, crGT TH:Cre^{+/-} rats with chronically downregulated DA showed significant improvements in decision making after a low dose of amphetamine. crGT TH:Cre^{-/-} rats overall risk-preference remained stable after amphetamine dosing. Asterisks denotes $p < 0.05$. Values are averages \pm SEM.

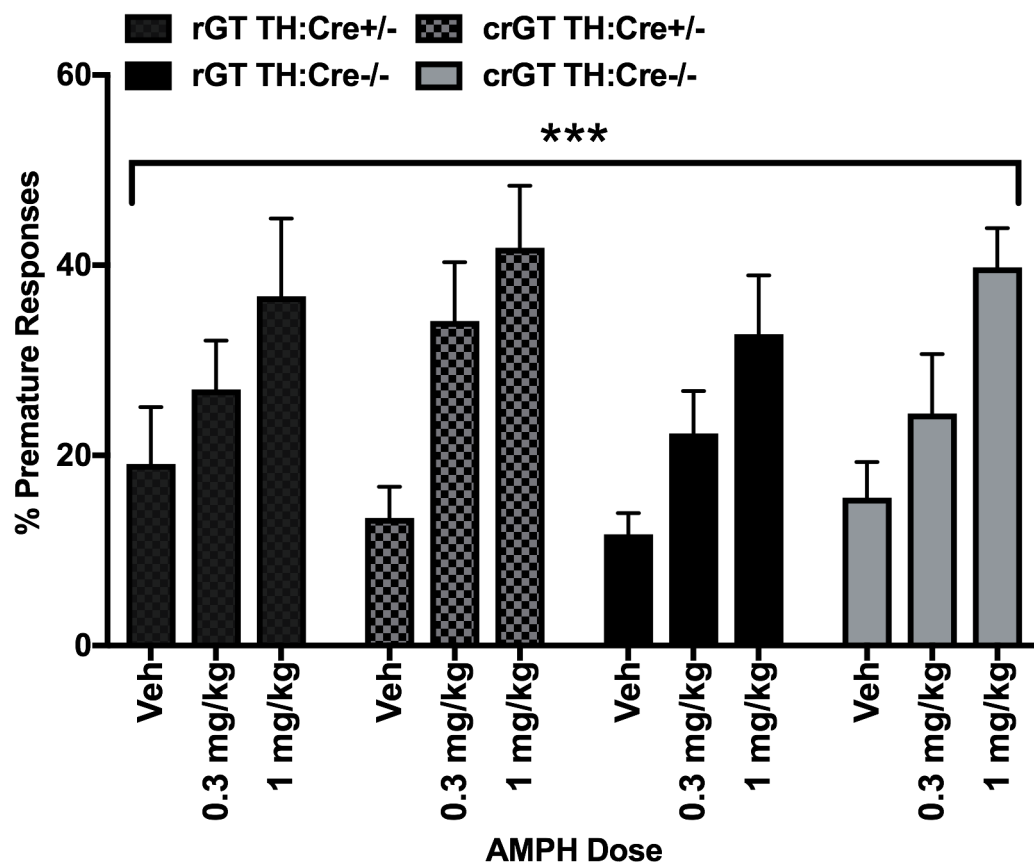


Figure 6-8 Percent premature responses during concomitant CNO and amphetamine administration.

All animals exhibited significant, dose dependent increases in behavioural disinhibition regardless of transgene or task experience. Triple asterisk depicts $p < 0.0001$. Values are averages \pm SEM.

Chapter 7: General Discussion

7.1 Summary of experimental findings

Here we show that decision making is a behavioural phenotype uniquely sensitive to drug-induced deficits, and baseline risk-preference confers greater susceptibility to these behavioural insults. Interestingly, adding reward-paired cues to the rGT resulted in lowered NAc DA release relative to uncued trained counterparts, potentially producing a reward deficient state both sensitive to the reinforcing effects of cocaine but also amplifying the negative sequelae of cocaine self-administration on choice. Furthermore, we demonstrate that DA efflux may play an important role in the expression of risky choice, data which point to potential targets for future research and therapeutics.

Experiment one demonstrated rats who had a natural preference for the risky options of on the rGT exhibited greater drug seeking, exacerbated risky choice, and potentiated incubation of craving after withdrawal. Choice impairments were also maintained during the withdrawal period, matching data seen in the clinical literature (Wang et al., 2012; Wang et al., 2013), and suggest these deficits may be the result of persistent neurobiological adaptations. Importantly, risk-preferring rats did not self-administer more cocaine than optimal counterparts, indicating worsened choice was likely due to a greater vulnerability in reward mechanisms which both underlie sensitivity to cocaine and decision making. We also found the changes in behaviour were limited to choice: impulsive responding was not worsened during self-administration, and only transiently increased during withdrawal, supporting the hypothesis that risky choice may

serve as a unique cognitive endophenotype for addiction. This model provides a clear behavioural target for future experimental and therapeutic interventions.

Experiment two sought to investigate whether cue-enhanced risky choice impacted acquisition of cocaine self-administration in crGT trained rats. In contrast to experiment one, all animals, regardless of decision making profile, showed rapid and robust acquisition of cocaine self-administration. Furthermore, all cocaine-experienced rats showed worsened decision making during concurrent diurnal crGT sessions, indicating the crGT cultivates changes to neurocircuitry imperative to decision making and sensitivity to drugs of abuse. Importantly, changes in choice preference did not significantly correlate with amount of drug consumed. Interestingly, optimal but not risk-preferring cocaine rats showed improvements in impulse control, suggesting drug exposure distinctly impairs decision making, but may even alleviate behavioural disinhibition, consistent with previous work (Caprioli et al., 2013). Finally, a subgroup of animals underwent a 30-day withdrawal period during which task performance was monitored, and we found, reassuringly, that cognitive deficits brought on by cocaine were somewhat alleviated in optimal animals. These data suggest that risky choice remains particularly vulnerable to drug-induced changes, but may be reversed in individuals who acquire adaptive choice strategies.

Experiment three investigated the influence of risky choice and task experience on the mesostriatal DA pathway. Previous work found that repeated exposure to uncertain outcomes and cues which predict probabilistic rewards sensitize the locomotor response to amphetamine (Singer et al., 2012; Zack et al., 2014). To determine whether experience with the uncertain options of rGT and crGT produced a similar effect, cocaine-induced ambulation was assessed prior to and after task training. Surprisingly, crGT rats locomotor activity was unchanged after task training compared to rGT trained rats, a difference only exhibited after task experience. Also

unexpected were the lack of differences by task and risk-preference in responding for CRf. These data suggest that risky choice on the crGT is *not* driven by the tendency to respond for reward-paired cues *per se*, and also that the cocaine self-administration profiles observed in experiments one and two are the result of potentiated sensitivity to cocaine rather than enhanced operant output for reward-predictive cues. To interrogate the involvement of NAc DA in the aforementioned locomotor activity effect, microdialysis probes sampled dialysate from the border of the NAc shell and core. HPLC analyses found that crGT trained rats exhibited significantly less tonic NAc DA release compared to rGT animals, but a slightly greater phasic response after cocaine. These results collectively suggest crGT training may cultivate a hypodopaminergic state within the VTA to NAc pathway, potentially resulting in greater sensitivity to the risky options of the crGT, drug, and cocaine-induced cognitive deficits. Although crGT performance is associated with greater risky choice, we were unable to conclude that less NAc DA resulted in increased preference for the disadvantageous options of the task, as the effect of task dominated any effect of basal decision-making preference.

Based on the results from study three, the final experiment assessed whether blunted NAc DA would be sufficient to precipitate risky choice. Using chemogenetic methods, TH:Cre rats were transfected with Cre-dependent inhibitory DREADD hM4D(Gi) into the same NAc area where dialysates were collected previously. Confirmation of viral expression found the vast majority of VTA afferents to these coordinates innervate the NAc shell, a subregion critical for responding to reward magnitude and cues to influence behavioural output (Beyene et al., 2010; Stopper and Floresco, 2011). DA release in the shell tracks cues encoding reward sizes (Sackett et al., 2017), and is thought to be aberrantly recruited in addiction (Koob, 2003). Acute and chronic downregulation of NAc DA improved decision making on both tasks, indicating even if

the rat exhibits an overall optimal strategy, DA efflux fosters a tolerance to risk. When systemic DA was potentiated by a low dose of amphetamine, improvements in choice were enhanced in crGT rats, whereas the high dose of amphetamine impaired performance in all animals. These data indicate there is a fine balance between DA tone in the NAc and other mesocorticolimbic structures which may promote-- or dampen-- biases in decision making. These results suggest it is likely an interactive network rather than the NAc alone which promote the expression of suboptimal choice.

7.2 Theoretical implications, and predictions for future studies

Together the results presented in this work suggest decision making is a key cognitive facet susceptible to drug-mediated changes, and demonstrates DA activity within the NAc may serve as an important neurobiological locus for decision making and addiction vulnerability. These works provide pivotal evidence for the role of disadvantageous choice in addiction, and set the stage for additional testable hypotheses. Future experiments may shed light on the contribution of other cortical and subcortical structures to risk-preference and drug-induced deficits. The prospective studies described in this discussion would be suitable for completion in the supervisor's laboratory.

7.2.1 Individual differences in dopamine receptor expression, and manipulation of receptor activity

It is clear from the data DA efflux influences decision making, but these results do not speak to the contribution of the DA receptor family in choice preferences. It would be imperative to explore whether risk-preferring or optimal animals have distinct expression profiles of D₂-like

receptors within the mesocorticolimbic network. We have previously found modulation of D₃ receptor activity affects choice on the crGT (Barrus and Winstanley, 2016), and a D₂ antagonist improved rGT performance ((Zeeb et al., 2009); but see also (Di Ciano et al., 2015)). Modulation of D₂ activity within the NAc and BLA also mediates performance on a probabilistic discounting task (Stopper et al., 2013; Larkin et al., 2016). Using western blots and real-time qPCR, measuring DA receptors within the NAc, BLA, and PFC could reveal fundamental differences between risk-preferring and optimal animals trained on the rGT and crGT. These techniques could also be used to determine any changes in receptor expression after drug experience. Another available option would be to over-express D₂ receptors within the NAc using viral mediated gene transfer prior to task acquisition to determine whether basal expression influences the likelihood to become risk-prone or averse (for example see (Gallo et al., 2015)). Such studies would further our understanding as to why some rats might be more sensitive to the phasic increases in dopamine, either by cues, reward size, or cocaine.

Modulation of DA D₂ receptor activity within the NAc at baseline may also help us understand the involvement of this subfamily in choice biases. To our knowledge, there have been no experiments which investigate NAc D₂ or D₃ involvement on the rGT or crGT. The simplest approach for a follow-up experiment would be to surgically implant cannulae above the NAc shell or core and deliver D₂ and D₃ selective agents prior to task performance. Alternatively, such manipulations during the acquisition of self-administration may promote or prevent cocaine-induced cognitive deficits, and should be explored.

7.2.2 Investigating the dissociable roles of the PFC and BLA inputs to the NAc in decision making

As previously stated, a breadth of data has implicated the PFC and BLA as important mediators of decision making (St Onge and Floresco, 2010; Zeeb and Winstanley, 2011, 2013; Larkin et al., 2016; Jenni et al., 2017). For example, the mPFC has been implicated in optimizing choice (Zeeb et al., 2015) and lesioning the BLA potentiated risk-preference (Zeeb and Winstanley, 2011) on the rGT. These regions and their projections to the NAc are also susceptible to drug-induced physiological changes (Stalnaker et al., 2006; Calu et al., 2007; Stalnaker et al., 2007a; Stalnaker et al., 2007b; Lucantonio et al., 2012; Lee et al., 2013; Ma et al., 2014). Furthermore, both regions have been found to mediate DA release within the accumbens (Floresco et al., 1998; Quiroz et al., 2016). Modulating inputs from the subregions of the PFC (namely the ventral mPFC, prelimbic and infralimbic cortices) and BLA to the NAc would provide significant insight as to the contribution of these projections in risky choice.

Newer chemogenetic technologies provide the means to transfect specific pathways implicated in task performance. Using a dual virus approach, a Cre-containing canine virus (CAV2-Cre) is infused into the terminals of the target projection (i.e. the NAc) and the Cre-dependent DREADD is delivered into the cell body of the afferent neuron (e.g. AAV-hSyn-DIO-hM4D(Gi) into the BLA). Due to the retrograde nature of CAV2-Cre, the DREADD will be exclusively expressed in neurons projecting from the BLA to the NAc and can be selectively silenced using systemic CNO administration (Boender et al., 2014). Furthermore, groups have developed new DREADDs which respond to ligands other than CNO, including the inhibitory kappa opioid receptor DREADD (KORD) which is activated by salvinorin B (Marchant et al., 2016b; Marchant et al., 2016a). By employing a “multiplex” DREADDs approach using the

CAV2-Cre system, KORD and hM4D(Gi) can be delivered into the mPFC and BLA, respectively, and projections to the NAc can be silenced individually or in concert via systemic CNO and/or Salvinorin B challenges. Such an approach would allow us to examine the influence of dynamic top-down and bottom-up NAc inputs in decision making.

7.2.3 Optogenetic interrogation of DA efflux during task epochs

It is unclear from the current data whether NAc DA release during wins, losses, or even choice influences risk-preference. Previous results using in vivo fast-scan cyclic voltammetry have found NAc DA is specifically released in response to wins and cues (Beyene et al., 2010; Sugam et al., 2012; Sugam et al., 2014; Sackett et al., 2017), whereas dips in efflux occur after loss of expected reward (Yacubian et al., 2006; Schultz, 2016b). Manipulating DA release within the NAc during discrete task epochs would further our understanding as to *when* efflux may affect choice. Optogenetics provides a powerful tool by which we can modulate neuronal activity in “real time.” Inhibitory opsins, including halorhodopsin, and archaerhodopsin (Arch), are extremely useful in determining the involvement of a region or a projection in a behaviour (Yizhar et al., 2011). When expressed in TH:Cre rats, we can decrease dopaminergic activity during particular epochs of the rGT and crGT (Witten et al., 2011). In addition to modulating DA activity at baseline, identification of a particular epoch to target would allow us to inhibit DA during self-administration sessions to prevent the exacerbation of decision making seen in experiments one and two.

7.2.4 Alternative neurotransmitter systems

Although previous work and the data presented here implicated DA in decision making and SUD, the expression of the addictive phenotype is likely the product of several neurochemical systems modulating reward processing and choice. One potential candidate would be corticotropin releasing hormone (CRH), activated in response to stress (Koob, 2010). Some evidence suggests drugs of abuse activate the CRH, and withdrawal may be the result of not only abstinence, but adaptations in “anti-reward” circuitry consisting of the central amygdala and NAc shell (the “extended” amygdala), which offset the reinforcing properties of drugs (Koob and Le Moal, 2005). Indeed, inhibition of CRH receptor activity mitigates compulsive drug seeking, although this effect is more prominent for opiates and alcohol (Schulteis and Koob, 1994; Koob, 2010). Acute stress also appears to potentiate disadvantageous choice on the IGT (Wemm and Wulfert, 2017). Exploring whether acute or chronic stress or CRH activation influences rGT and crGT performance warrants further investigation.

Recently, serotonin has also been implicated in maintaining drug abuse. Indeed, differential modulation the 5HT_{2C} receptor influences responding for cocaine-paired cues (Anastasio et al., 2014) and drug rewards (Cunningham et al., 2011). 5HT_{2A} and 5HT_{2C} receptors act antagonistically within the PFC to influence impulse control, where 5HT_{2A} activation potentiates motor impulsivity but 5HT_{2C} curtails it (Anastasio et al., 2015). We have recently found that systemic administration of a 5HT_{2C} receptor antagonist improved decision making while impairing impulse control on the crGT (Adams et al., 2017), an intriguing result when considering the dissociation in optimal rats’ crGT performance after cocaine self-administration: increased risky choice but decreased motor impulsivity. Furthermore, systemic 5HT_{1A} agonist 8-OH-DPAT increased choice of P3 on the rGT (Zeeb et al., 2009). Interrogating the influence of

serotonergic activity in the NAc, amygdala, and PFC would also expand our understanding of the neural mechanisms which contribute to risk-preference.

7.3 Limitations and critical considerations

The rGT and crGT are powerful tools to assess cost/benefit decision making, and when used in combination with self-administration, allowed us to determine whether decision-making preferences are related to drug seeking. While the data presented here closely mimic those seen in the clinical literature, there are critical limitations to consider when interpreting the results and considering future study design.

The first limitation is the lack of acknowledgement of potential sex differences. We exclusively used male rats in these studies to be consistent with previous work from the Winstanley laboratory. However, there are considerable sex differences in both addiction and decision-making performance. In the clinical population, females are significantly more likely than males to develop an addiction, escalate drug use, and relapse (Becker, 2016). Furthermore, there are distinct sex differences in drug preference: males tend to develop alcohol and heroin abuse, whereas females show a greater sensitivity to psychostimulants (Becker, 2016; Becker and Koob, 2016). Previous data from the IGT and an rGT variant show females have impaired task performance (de Visser et al., 2011; Singh, 2016). At the neurobiological level, females have significantly greater NAc DA efflux, which is tempered by gonadal hormones (Cummings et al., 2014; Becker, 2016). Unfortunately, too few research studies take these differences into account or study their influence on treatment prospects. The National Institutes of Health recently set a mandate to include both male and female subjects in scientific studies. Future research should certainly incorporate not only the inclusion of female subjects, but also pay close

attention to sex differences in behavioural and neurobiological contributions to decision making and drug-induced cognitive deficits.

Another critical consideration of the data is the timepoint in which behaviour is assessed. Each study presented here examined behaviour at baseline, after decision-making preferences had been acquired. However, dopaminergic activity is actively recruited during the acquisition of stimulus-outcome and action-outcome associations (Fiorillo et al., 2003; Schultz, 2007), and although downregulation of DA at baseline improved choice, interrogating its role in the “exploration” phase of the rGT and crGT may help us understand whether the formation of choice biases depend on DA.

Thirdly, the influence of training length on behaviour should be noted. Animals are trained on the rGT and crGT for approximately 30 sessions prior to experimental manipulations. A recent meta-analysis showed rats as early as session 10 exhibit primarily optimal decision making on the rGT (Barrus et al., 2015b). Due to the reliance of behavioural stability to confirm external manipulations are in fact changing cognitive performance, there remains the question whether decision making seen at baseline is perseverative or deliberative in nature, although previous work suggests the latter (Zeeb and Winstanley, 2013). This distinction is of particular importance for interpretation of behaviour seen experiment 2 (chapter 4), in which crGT risk-preferring animals exhibited potentiation of P3, their most preferred option. This is somewhat in keeping with addiction literature, which suggests drugs like cocaine result in “inflexible” neuronal activity which hinders the animal’s ability to update behaviour in response to changes in contingencies (Stalnaker et al., 2006; Stalnaker et al., 2007a; Stalnaker et al., 2007b). However, cocaine-exposed optimal rats did not show simple enhancement of their baseline preference, but acquisition of maladaptive choice. Likewise, risk-preferring rGT animals did not

show potentiation of a single option, suggesting the increases in risky decision making are due to altered valuation of multiple outcomes. Regardless, it would be imperative for future experiments to determine whether deficits in decision making are simply the perseveration of choice or represent changes or enhancement of value for the disadvantageous options of these tasks using inter-trial analyses of behaviour (i.e. win/stay, lose/shift).

Related to the issue of interpretation of decision making changes, when examining shift in individual choice, our results infer animals are valuing the risky or safe options more after manipulations. However, alternative explanations may account for changes in decision making. For example, greater selection of P1 is not inherently “disadvantageous”, as the net gain across a session, although less than that of P2, is still greater than P3 and P4. Therefore, choice of this option might not reflect impaired decision making, but rather greater sensitivity to punishing time-outs, or general risk aversion. Similarly, selection of P4, while clearly maladaptive, may not necessarily indicate “myopia for the future”, but rather insensitivity to the delay or even impairments in working memory. Including other assays of reward sensitivity, tolerance to delay, and working memory would help to elucidate interacting psychological and neurobiological processes which promote the cognitive impairments seen in these studies.

Experiments one and two strove to investigate the involvement of individual differences in decision making in addiction risk, a critical research question. Results from these studies echo those seen in the human literature, giving these data exceptional translational value. However, these experiments relied upon the natural expression of risky decision making, and due to cohort variability and technical demand of these studies, required substantial replication to achieve sufficient power for behavioural analyses. Indeed, experiment one (chapter 3) extracted risk-preferring animals from a total of four separate cohorts, and due to length of training, required

nearly two years of experiments to achieve adequate subject numbers. A major criticism of the study of individual differences is that although they may elucidate key contributors to psychopathologies, they are particularly costly, both financially and timewise. Previous work has found cognitive deficits may be heritable, such as impulsivity (Jupp et al., 2013), which may be an option for exploring genetics and suboptimal choice. But, this avenue may require generations of breeding to achieve optimal expression of disordered phenotypes within a normal cohort. Determining ecologically valid ways to increase the number of risky rats within a cohort would be massively helpful for the study of impaired decision making in addiction and other psychiatric disease.

Finally, the results presented here are collected from rats using a model with remarkably high face, construct, and predictive validity (de Visser et al., 2011), and may allow for translation to the clinical population. Although many clinical studies have noted maladaptive choice in addicted subjects, to the candidate's knowledge, none have attempted to treat poor decision making as a symptom. Studies which employ a dual preclinical and translational approach using the rGT and IGT may help to identify pharmacological or behavioural interventions for SUD.

7.4 Concluding Remarks

In sum, using a validated rat analogue of the IGT used clinically, we have shown decision making is uniquely susceptible to impairments after cocaine exposure. Furthermore, preference for risk precedes drug exposure, and is associated with greater responding to drugs of abuse and relapse. Salient win-paired cues, akin to those used in common gambling devices, cultivate changes in accumbens dopaminergic efflux, which is associated with risky choice, vulnerability to drug seeking, and cocaine-induced choice impairments. Experimentally reducing NAc DA also helped to limit tolerance of the risky options for both tasks. These data indicate aberrant dopaminergic signalling fosters cognitive impairments which may promote the addicted state. Future exploration of factors which promote maladaptive decision making may help to prevent and treat addiction, and hopefully will reframe the perception of SUD as a treatable condition rather than a “lack of willpower” syndrome.

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