TOWARDS A BETTER UNDERSTANDING OF THE BIDIRECTIONAL RELATIONSHIP BETWEEN DECISION-MAKING AND ADDICTION VULNERABILITY

by

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Abstract

Impaired decision-making is recognized as a diagnostic criterion for many different psychiatric disorders including gambling disorder and substance use disorders. Habit formation and cue responsivity are known to play a role in driving these disorders, but less is known as to if and how decision-making is differentially altered by different classes of abused drugs. The impact of biological sex is also vastly understudied in such contexts. To gain further understanding on the bi-directional relationship between decision-making impairments and addiction, we combined the well-established rat gambling task with volitional drug self-administration in both male and female rats. In one aim, I sought to pharmacologically manipulate habit formation via the systemic administration of a histone deacetylation inhibitor. I found that inhibiting histone deacetylation resulted in increased risky choice during acquisition of our rat gambling task, while also altering how rats were learning from task outcomes. In another aim, I sought to further characterize the effect of cues on decision-making by allowing rats to choose between the cued and uncued rat gambling task on a trial-by-trial basis. I found that cues had negative effects on cognition, despite being preferred to their absence. I then paired this task with cocaine self-administration to investigate the bi-directional effects of gambling and cocaine-taking. I found evidence for cocaine-induced motivational deficits on subsequent gambling sessions. In my final aim, I sought to determine whether fentanyl could induce the same decision-making impairments that we had previously observed in response to cocaine. I found that while acute fentanyl self-administration did not impact cognition, fentanyl withdrawal did have long-lasting negative effects on decision-making.
Lay Summary

Understanding how decision-making impairments are bi-directionally related to gambling and addiction is critical to informing the successful treatment of these disorders. In attempt to gain further understanding on the intricacies of this relationship, I conducted a series of experiments aimed at manipulating habit formation, presence of cues, and access to multiple different drugs of abuse in rats performing the rat gambling task. I found that cues have detrimental effects on cognition, and that histone acetylation may play a role in this relationship. I also found that different drugs of abuse have differential effects on cognition. Overall, these findings add to existing literature indicating that cognitive differences such as risky decision-making can be both a cause and consequence of drug abuse, and that pre-existing decision-making strategies can influence response to and for these drugs as a factor of biological sex.
Preface

Chapter 3 is currently being prepared for publication under the working title “Histone deacetylase inhibitor sodium butyrate increases risk-taking during acquisition of the rat gambling task,” by K.M. Hrelja, S. Hipkin, V. Domah, B. Hathaway, C. Chernoff, and C.A. Winstanley. KH, SH, and CAW designed the experiments. KH and CAW co-wrote the manuscript and made the majority of intellectual contributions. KH, SH, and VD collected the behavioural data. KH performed histology and conducted statistical analyses. KH and CC performed microscopy, and BH conducted computational modelling of the behavioural data.

Chapter 4 is being prepared for publication under the working title “Examining the interaction between gambling, drug-taking, and decision making using a novel behavioural task,” by KMH, C. Hales, S. Ansary, E. Chong, B. Russell, & CAW. KMH and CAW designed the experiment. KMH and CAW co-wrote the draft manuscript and made the most intellectual contributions. KMH, SA, EC, and BR collected the behavioural data. KMH performed the surgeries. KMH conducted statistical analyses and CH performed computational modelling of behavioural data.

Chapter 5 has been submitted for publication under the title “Increased risky choice during forced withdrawal from fentanyl on the cued rat gambling task,” by KMH, C. Kawkab, D. Avramidis, S. Ramaiah, and CAW. KMH and CAW designed the experiments and made the majority of intellectual contributions. KHM, CK, and CAW co-wrote the manuscript. KMH, CK, DA, and SR collected behavioural data. KMH performed the surgeries and conducted statistical analyses.
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 number A21-0012.
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Dedication

As someone who has loved ones caught in the cycle of addiction and who has personally witnessed their struggle to achieve even the most basic of necessities, I dedicate this dissertation to all the individuals in the DTES community. I stand with you in Spirit and hope that my research can contribute to a better understanding of the cognitive basis and consequences of addiction, and thus inform treatment options for all of those who suffer from substance use disorders.
Chapter 1: General Introduction

1 in 5 Canadians meet the criteria for substance use disorder (SUD) in their lifetime (Statistics Canada, 2015), contributing to an economic burden of nearly 50 billion Canadian dollars per year (Canadian Substance Use Costs & Harms, 2020). Beyond financial costs, addiction can have devasting personal costs on not only individuals with addiction but on their loved ones as well. Thus, the effects of addiction are ubiquitous. Despite this, our understanding of addiction and viable treatment options for those who experience such disorders remain limited.

In order to yield more successful outcomes in the treatment of addiction, we must first gain a more thorough understanding of both the causes and consequences of drug abuse. Through the research presented in my dissertation, I aim to further our knowledge on the bi-directional relationship between decision-making impairments and addiction. I will first outline and discuss different schools of thought surrounding the etiology of addiction, from the contribution of habit vs goal-directed behaviour and their neurobiological underpinnings to the connections between neurocognitive differences and addiction-relevant behaviour.

1.1 Habit Formation and Goal-Directed Behaviour

Substance use disorders and behavioural addictions are both characterized by a loss of control over use or engagement despite negative effects in one or more life domains. Such disorders are related to changes in neural networks responsible for executive functioning and reward processing, and are associated with maladaptive habit formation as well as deficits in goal-directed behaviour (Antons et al., 2020; Vandaele & Janak, 2018).
Habit formation involves the incorporation of memories into long-term storage and may be defined as the process by which a behaviour becomes automatic. One defining feature of habits is their inflexibility, or resistance to degradation and change. Particularly, habits do not change in the face of altered reinforcer value (Quinn et al., 2013; Thrailkill et al., 2021). This may contribute to the maintenance of addiction as drug use continues despite the drug no longer producing the euphoria associated with initial drug use, which one may interpret as a diminished reinforcer value. Similarly, drug use continues despite rising negative consequences of use, which may be interpreted again as altering reinforcer value. Indeed, sensitivity to reinforcer devaluation is commonly used to assess addiction-relevant behaviour in animal models. Such studies often find that response for drug reinforcer is sensitive to devaluation after acute exposure, but insensitive following prolonged experience with that drug (Clemens et al., 2014; López et al., 2016; Zapata et al., 2010). This may be reflective of a shift from goal-directed to habit-mediated processes with increased drug exposure, and thus the shift to addiction. Likewise, it has been found that behavioural inflexibility and insensitivity to reinforcer devaluation can be used to distinguish which animals go on to become high vs low drinkers, indicating that predisposition to habit formation is indicative of addiction vulnerability (Merchán et al., 2019). In further support, it has been found that individuals with substance use disorders (SUDs) are quicker to achieve habit formation, even in non-drug related scenarios (McKim et al., 2016). Likewise, it has been shown that gambling speed and betting inflexibility, markers of habit formation, are both increased as one gains more experience in slot machine gambling (Ferrari et al., 2022). The pertinence of habit formation may thus be extended to behavioural addictions, though research in this field is still sparse. The neurobiology underlying habit formation will be discussed in the following section of this dissertation.
It should be noted that there is also evidence of the contrary – that drugs of abuse gain greater value over the course of addiction, and that it is thus an increase in goal-directed responses rather than habit formation which is responsible for the maintenance of addiction (Hogarth, 2020). This has been demonstrated as increase in drug choice over alternative reinforcers in both opioid and cocaine use disorder, as well as in nicotine dependence, and is exacerbated by negative affect (Chase et al., 2013; Hogarth et al., 2019; Moeller et al., 2018). Likewise, increased goal-directed behaviour is positively correlated to problem gambling scores, even after controlling for symptoms of depression, indicating that these results may be translated to behavioural addictions (Marchica et al., 2019). In animal models, increased progressive ratio responding is seen with increased exposure to drugs of abuse, serving as a model for goal-directed behaviour in addiction (Halbout et al., 2023; Puhl et al., 2009). Indeed, the lengths and strain individuals endure in order to acquire and take their drug of choice supports the contribution of excessive goal-directed behaviour, rather than mere habit, in active addiction.

More research is thus required to further parse out the contribution of altered habit formation and goal-directed behaviour to the maintenance of addiction. Of course, these two mechanisms aren’t necessarily acting in opposition. As dual-process theories would suggest, it may be the case that these two mechanisms contribute to the maintenance of addiction in parallel, with only one system dominating at any given time (Vandaele & Janak, 2018). With a deeper understanding of these cognitive mechanisms, it may be possible to improve treatment outcomes for those experiencing addiction. Unfortunately the rates of relapse remain high at 40-60% of abstinence leading to relapse depending on drug of choice, though some have reported relapse rates as high as 91% for those with opioid use disorders (National Institute on Drug
Abuse, 2020; Smyth et al., 2010). There is thus a clear need for the better understanding and treatment of addiction.

1.1.1 The Neurobiology of Habit Formation

In early learning, goal-directed behaviour dominates. The dorsomedial striatum (DMS), which receives dopaminergic input from the medial substantia nigra pars compacta (mSNc) and glutamatergic input from the orbitofrontal cortex (OFC), is thought to play a critical role in promoting this behaviour (Burton et al., 2015). Together, the DMS and OFC are also required for maintaining cognitive flexibility, which allows for the updating of reward value (Uddin, 2021; Z. Wang et al., 2020). This is particularly true for learning in the presence of cues (Ostlund et al., 2017). Indeed, lesioning of the DMS causes cognitive inflexibility, as measured by failure to perform reversal learning (Z. Wang et al., 2020) and insensitivity to reinforcer devaluation (Corbit et al., 2013), indicating that the DMS is required for goal-directed behaviour and that damage to this region causes the reliance on habitual behaviour.

The ventral striatum (VS), in which the nucleus accumbens (NAc) is located, receives input from the ventral tegmental area (VTA), insula, and medial prefrontal regions, is also known to play a role in motivated, goal-directed behaviour and cue-reward association learning (B. L. Goldstein et al., 2012; Haber, 2011; Tang et al., 2022). Importantly, the VS is also a key component of the brain’s reward circuitry, where it mediates the reinforcing properties of abused drugs via dopaminergic innervation (Haber, 2011; Wise, 2004).

As training on a task progresses, there is a shift to the habitual control of behaviour. This is thought to be largely mediated by the dorsolateral striatum (DLS), which receives dopaminergic input from the lateral SNC and glutamatergic input from the lateral OFC (Burton et
al., 2015). Thus, there is a shift from DMS to DLS control over the course of learning, and this marks a shift to reliance on habit (Cataldi et al., 2021; Yin et al., 2009). Indeed, increased activity in the DLS is seen while engaged in a task that has been overtrained, and is inversely correlated to time spent deliberating on an action (Smith & Graybiel, 2013). Likewise, increased DLS activity is seen during the execution of well-learned (habitatual) motor sequences (Rueda-Orozco & Robbe, 2015). Similar to how disruption of the DMS accelerates habit formation, disruption of the DLS causes a shift back to goal-directed behaviour (Jonkman et al., 2012; Turner et al., 2022). For example, lesioning the DLS results in the re-emergence of goal-directed behaviour, even after prolonged training (Lingawi & Balleine, 2012).

These regions are similarly implicated in addiction, where a shift from prefrontal to striatal control is seen as drug use becomes habitual (Everitt & Robbins, 2005). Specifically, prefrontal regions including the OFC become dysregulated in addiction, resulting in the suppression of executive functions and goal-directed behaviour (R. Z. Goldstein & Volkow, 2011; Schoenbaum & Shaham, 2008). Simultaneously, there is a shift from ventral and dorsomedial striatal to dorsolateral striatal control, which promotes the reliance on habit and thus the maintenance of addiction (Everitt & Robbins, 2005, 2013). Indeed, nicotine self-administration has proven to be insensitive to reward devaluation under extended, but not acute, self-administration periods, and this insensitivity to reinforcer devaluation was accompanied by increased activation of the DLS and SNc (Clemens et al., 2014). Similarly, habitual alcohol-seeking following acquisition of self-administration has been shown to rely on glutamatergic and dopaminergic signaling within the DLS (Corbit et al., 2014).

While the transition from goal-directed to habitual control relies largely on dopaminergic signals from the mSNc to DMS and from the lSNc to DLS respectively, this may be mediated by
cholinergic interneurons (CINs) in striatal subregions. Indeed, the inhibition of CINs in the dorsal striatum has been shown to cause a reduction in DMS but not DLS dopamine levels, which impairs cognitive flexibility and causes insensitivity to reinforcer devaluation (Favier et al., 2020). This indicates that the depletion of acetylcholine dysregulates dopamine levels in the DMS to promote habit formation.

1.2 Theories of Addiction

There are numerous theories regarding what causes and contributes to the maintenance of addiction, beyond the contribution of maladaptive habitual and goal-directed behaviour. It was once thought that individuals continued to abuse drugs despite rising negative consequences in order to avoid withdrawal symptoms. Certainly, one can see how this may contribute to the maintenance of an opioid use disorder, where withdrawal symptoms include unrelenting body pains, diarrhea, rhinorrhea, nausea/vomiting, insomnia, yawning, and sweating, amongst a myriad of equally unappealing cognitive symptoms. However, this theory fails to account for e.g. cocaine use disorder, which causes much less severe withdrawal symptoms over a much shorter time scale. It also fails to explain why many individuals relapse following successful detox, when physiological withdrawal symptoms are no longer being experienced.

Another theory aimed at explaining addiction is the positive reinforcement theory, which posits that individuals abuse drugs in order to achieve the euphoric effects that they provide. However, the initial euphoria produced by these drugs quickly subsides while drug use persists. Soloman’s opponent process theory of addiction builds off this observation. According to this theory, while individuals initially use drugs for their euphoric effects, this euphoria is replaced by a state of negative affect, which drives continued use (Solomon & Corbit, 1974). In other
words, these individuals come to rely on drugs in order to feel good. In support of this theory, hyperkatifeia, or the heightened experience of negative emotions, is indeed evident during withdrawal from drugs of abuse, and is much longer lasting than physical withdrawal symptoms (Koob, 2021). Indeed, the surgical removal of the anterior cingulate cortex (ACC), responsible for the regulation of emotions, has proven to be a remarkably effective, albeit drastic, treatment for addiction (Kanaka & Balasubramaniam, 1978; Stelten et al., 2008).

Another school of thought includes the incentive sensitization theory, which states that drug-paired cues gain incentive salience which then drives drug-seeking and taking. Indeed, this theory better explains why individuals frequently relapse upon returning to their drug-paired environment. This theory was popularized by Berridge and Robinson, who further demonstrated long-lasting changes in dopaminergic signaling to be a mediating factor in excessive, cue-induced wanting of drugs (Berridge & Robinson, 2016). Indeed, numerous studies have shown that addiction is linked to increased responsivity to drug-paired cues (Al-Khalil et al., 2021; Karoly et al., 2019; Kühn & Gallinat, 2011). Yet, other studies demonstrate that cue reactivity is not reliably linked to dependence severity in human addiction (Guterstam et al., 2022; Karoly et al., 2019; Perkins, 2012).

Similarly, the reward deficiency theory posits that addiction is driven by low basal levels of dopamine. This theory then suggests that addicts seek out the dopamine rush provided by drugs of abuse in order to compensate for their inherent lack of dopaminergic tone. This theory was popularized by Kenneth Blum, whose group has also shown that alterations in D2 dopamine receptors lead to excessive reward seeking (Blum et al., 1996). Other groups have since found evidence further supporting the idea that addiction to various different drugs of abuse are linked
to deficiencies in dopaminergic signaling (Burns et al., 2019; Hou et al., 2014; Martinez et al., 2012).

With so many theories demonstrating considerable support, perhaps a better view is that these theories are not mutually exclusive, and that a combination of these views, in addition to genetic, environmental, and neurocognitive risk factors all contribute to the acquisition and maintenance of addiction. Furthermore, addiction disorders may be underpinned by different mechanisms in different individuals, or a combination of neurobiological factors interacting with one’s unique environmental exposures. The contribution of neurocognitive factors, specifically risky decision-making, will be discussed at length in section 1.3.

1.2.1 Comparing the Mechanism of Action of Stimulants Versus Opioids

All rewards cause an increase in dopaminergic signaling. Specifically, dopamine release from the VTA into the NAc occurs following natural rewards such as sex or food (Becker et al., 2001; Hernandez & Hoebel, 1988). However, this dopamine release is increased exponentially in response to drugs of abuse, with stimulants like amphetamine resulting in the largest increase in dopamine efflux (Di Chiara & Imperato, 1988; Hernandez & Hoebel, 1988). These artificially high levels of dopamine cause compensatory changes in the brain, such as the shift from prefrontal to striatal control as mentioned in section 1.1.1. Yet, different classes of drugs of abuse alter dopaminergic signaling in different ways, and can be classified as being via direct or indirect means. An overview of the mechanism of action for drugs of abuse relevant to this dissertation are depicted in Figure 1.

Stimulants such as cocaine directly impact dopamine functioning via the blockade of dopamine transporters, resulting in dopamine having longer action in the synapse (Trifilieff &
Martinez, 2014). Cocaine use has been shown to result in a variety of cognitive deficits, with the most omnipresent finding being decreased inhibitory control as a result of decreased activity in the ACC and PFC (Hester & Garavan, 2004; Kaufman et al., 2003; Trifilieff & Martinez, 2014). It has also been repeatedly demonstrated that stimulant use results in impaired decision-making, characterized specifically by an increase in risky choice (Ferland & Winstanley, 2017; Rogers, 1999). There is also reliable evidence of cocaine use causing pronounced deficits in working memory, which resolve with cessation of drug use (Vonmoos et al., 2014).

In contrast, opioids increase dopamine indirectly. Opioids inhibit GABAergic interneurons in the VTA, resulting in the disinhibition of dopaminergic neurons and thus the increase in release of dopamine into the NAc (Johnson & North, 1992). Despite acting via the same mechanism, individual opioids have important differences. For example, fentanyl is a fast-acting synthetic opioid that is 100 times stronger than the opiate morphine, and 50 times as strong as the opiate heroin. Fentanyl is also cheaper to produce and more addictive than opiates such as heroin, and thus poses a uniquely high risk for abuse and overdose. As with cocaine use, the use of opioids is associated with a myriad of cognitive deficits. Deficits in attention, working memory, and executive function have all been noted as a result of opioid dependence (Ornstein, 2000; Rapeli et al., 2006; Rogers, 1999). Interestingly, and in contrast to the effects of cocaine, it seems as though the most pronounced opioid-induced cognitive deficits actually arise during withdrawal from opioid use (Dalley et al., 2005; Jamison et al., 2003; Rapeli et al., 2006; Wheeler et al., 2023). Indeed, researchers have noted marked deficits in working memory, executive function, and fluid intelligence during early abstinence from opioids, likely resulting from PFC dysregulation (Rapeli et al., 2006). Likewise, long-lasting impairments in decision-making are seen during abstinence from fentanyl (Wheeler et al., 2023). The differences in
mechanism of action and drug-induced cognitive deficits between stimulants and opioids, and the salience of these deficits during opioid withdrawal, may thus lend insight into why relapse rates remain much higher for opioids than for stimulants like cocaine, and may thus present a target for therapeutic intervention during abstinence.

![Figure 1. Mechanism of action for fentanyl and cocaine.](image)

### 1.3 Neurocognitive Risk Factors for Addiction

As discussed in section 1.2.1, a range of cognitive deficits are known to arise as a result of chronic drug use. However, it is also known that similar cognitive deficits can precede initial drug use and may thus act as risk factors in the etiology of addiction. Recognized neurocognitive risk factors in addiction vulnerability include increased impulsivity, cognitive inflexibility, risky decision-making, novelty-seeking, reward sensitivity, reward learning, aggression, and having a
concurrent neuropsychiatric disorder (Christensen et al., 2023; Rose et al., 2019). The interactions between risky decision-making and addiction vulnerability will be discussed at length in later sections of this dissertation.

One prominent risk factor for the development of addiction is high trait levels of impulsivity, which may be defined as the tendency to act with little to no forethought. Indeed, it has been shown that individuals with cocaine and opioid dependence display impulse control deficits under delay-discounting paradigms (Coffey et al., 2003; Madden et al., 1997). Likewise, there is evidence for response inhibition deficits in those with pathological gambling (Odlaug et al., 2011). Further, preclinical studies have found that pre-existing impulse control deficits predicts escalating response for cocaine, increased responding for cocaine under punishment, and increased risk of relapse in rats (Dalley et al., 2007; Economidou et al., 2009; Everitt et al., 2008). Interestingly, pre-existing impulse control deficits do not seem to confer vulnerability to addiction of heroin in rats (McNamara et al., 2010), indicating that the potency of trait impulsivity as a risk factor for addiction may be drug-dependent. There is also a wealth of evidence to suggest that drugs of abuse are independently capable of inducing impulse control deficits (Jentsch, 2001; Simon et al., 2007). Likewise, impulse control disorders such as intermittent explosive disorder, kleptomania, and pathological gambling, are known to be highly comorbid with SUDs (L. Schreiber et al., 2011). Thus, there exists a complex bi-directional relationship between impulsivity and addiction vulnerability.

High levels of cognitive inflexibility, defined as the inability to alter behaviour in response to change, is another predictor of addiction vulnerability. Cognitive inflexibility has been noted in chronic cocaine users as well as those with pathological gambling (Ersche et al., 2008; Odlaug et al., 2011). Likewise, cognitive inflexibility is known to predict addiction
relevant behaviours in animal models. For example, cognitive inflexibility predicts faster acquisition of cocaine self-administration, as well as greater levels of both cocaine- and alcohol-taking (Cervantes et al., 2013; Loos et al., 2013). Yet, as with impulsivity, deficits in cognitive flexibility are also known to arise in response to exposure to drugs of abuse. Indeed, prior experience with both alcohol and cocaine have been shown to cause set-shifting and reversal learning impairments, respectively – both of which are indicative of cognitive inflexibility in these animals (Gass et al., 2014; Jentsch, 2002). Similarly, adolescent opioid exposure has been shown to induce cognitive inflexibility in adult female, but not male, mice (Wakhlu et al., 2023). This presents further evidence of bi-directional causality between neurocognitive deficits and drug abuse.

Increased sensitivity to reward and reward learning likewise confers increased risk of developing an addiction. Those with heightened sensitivity to reward are thought to be more attuned to the reinforcing effects of drugs of abuse, and are thus more prone to the development of addiction (Bijttebier et al., 2009; Loxton & Dawe, 2006; Loxton & Tipman, 2017). Indeed, it has been shown that increased reward bias predicts later alcohol, tobacco, and cannabis use at subsequent follow up (Van Hemel-Ruiter et al., 2015). Furthermore, those with SUDs have increased attentional capture by reward-paired cues, even in non-drug related tasks (Albertella et al., 2019), indicating that exposure to drugs of abuse may further alter reward processing. The influence of reward-paired cues on addiction vulnerability and decision-making will be further discussed in section 1.4.

Finally, rates of comorbidity between various neuropsychiatric disorders are high, indicating that having any other neuropsychiatric disorder may confer addiction vulnerability. Indeed, there is evidence to indicate that as many as 75% of individuals with a diagnosed
neuropsychiatric disorder have at least one other diagnosis, though true rates may be even higher (García-Marchena et al., 2018; Hser et al., 2001; Patron et al., 2022; Ross & Peselow, 2012). For example, it has been reported that 44% of those with major depression (Miller et al., 1996), 46% of those with post-traumatic stress disorder (Pietrzak et al., 2011), and 61% of those with bipolar disorder (Regier, 1990), have a comorbid SUD diagnosis. Likewise, it has been reported that up to 60% of problem gamblers meet the diagnostic criteria for a SUD (Barnes et al., 2015; Lorains et al., 2011; Rush et al., 2008). This could be attributed to shared risk factors such as impulse control and decision-making deficits amongst diagnoses, a causal relationship between diagnoses, or attempts at self-medicating. Furthermore, polysubstance use is rampant in the SUD landscape, making it difficult to disentangle the cognitive effects of specific drugs of abuse in humans. Overall, this indicates that many risk factors for addiction vulnerability are shared between different drugs of abuse and other neuropsychiatric disorders. Yet, models of concurrent disorders are underutilized in preclinical studies.

1.3.1 The Intersection of Risky Choice and Addiction

It is indisputable that increased crime is seen amongst individuals afflicted by addiction. Rates of criminal offense are 3-4 times higher in those that use drugs, with the sub-category of recreational drug users having lower offense rates than those who meet the diagnostic criteria for addiction (Bennett et al., 2008). Interestingly, elevated crime rates are also seen in individuals prior to the initiation of opioid use, when compared to individuals who do not go on to abuse opioids, and these rates are further exacerbated post-initiation of opioid use (Pierce et al., 2017). This also extends to behavioural addictions, with problem gamblers showing increased crime rates even after controlling for drug use (Grinols & Mustard, 2021). These data may suggest
underlying neurocognitive mechanism, such as decision-making impairments, linking criminal behaviour and addiction vulnerability. Furthermore, drug use is associated with increased unsafe decisions, such as the sharing of drug paraphernalia (e.g. pipes, syringes) and risk of overdose, yet those with addiction tend to underestimate the risks associated with these behaviours (Firestone et al., 2009; Frank et al., 2015; Rowe et al., 2016). Interestingly, it has also been found that greater use of opioids was correlated to lower perceived risk of overdose (Rowe et al., 2016), indicating that decision-making is increasingly impaired with increased drug use. These facts provide evidence of impairments in cost-benefit decision-making and increased risk-taking in those living with addiction.

Indeed, individuals with SUDs often have impaired decision-making, which can persist well into abstinence and negatively impacts multiple life domains (Ahn et al., 2014; Chen et al., 2020; Ersche et al., 2005). These decision-making impairments are also known to be correlated to treatment failure and addiction severity (Baldacchino et al., 2012; Fishbein et al., 2005). In support, a risky decision-making profile in the Iowa gambling task (IGT) has been seen in individuals with a dependence on various drugs, including marijuana and cocaine, and is known to predict relapse to such substances (Bolla et al., 2003; Stevens et al., 2013). Likewise, researchers have shown that opioid-dependent individuals similarly have impaired performance on the IGT (Lemenager et al., 2011). Additionally, longer periods of abstinence in methamphetamine-dependent individuals is associated with more optimal decision-making strategies on the IGT (G. Wang et al., 2013). There is also evidence that having concurrent disorders, such as meeting diagnostic criteria for both pathological gambling and SUD, is more detrimental in terms of decision-making deficits on the IGT than having one diagnosis alone (Krmpotich et al., 2015). However, it remains unclear whether decision-making deficits are a
cause or consequence of drug use due to various environmental and situational factors that are difficult to control for in human research.

In rodent studies, it has been demonstrated that increased levels of risky choice can predict addiction-relevant behaviours following first-time nicotine and cocaine exposure (Gabriel et al., 2019; Yates et al., 2021). Likewise, rats who have an inherent preference for large rewards despite high likelihood of punishment have been shown to self-administer more cocaine and show greater salience for reward-paired cues (Mitchell et al., 2014; Olshavsky et al., 2014). Conversely, it has been found that prior experience with multiple different drugs of abuse increases risk-taking in rats (Clark et al., 2012; Ferland et al., 2019; Ferland & Winstanley, 2017; Nasrallah et al., 2009; Wheeler et al., 2023). Evidence of increased risk-taking both before and after exposure to drugs of abuse points to a bi-directional relationship between decision-making deficits and addiction, which may be utilized to inform treatments for those with addiction.

While very few studies directly compare the effects of different drugs of abuse on decision-making, there is some evidence to suggest that impairments are comparable across multiple different drugs of choice, such as alcohol, cocaine, and opioids (Gowin et al., 2013). However, this is difficult to discern from human studies, as polysubstance abuse and comorbid neuropsychiatric disorders are omnipresent.

1.4 The Contribution of Cues to the Maintenance of a Disordered State

Drug-paired cues are known to facilitate addiction by promoting increased craving, drug-seeking, and drug-taking in both humans and rodents (Perry et al., 2014; Pitchers et al., 2018; Vafaie & Kober, 2022). These cues can be intrinsic (e.g. emotional states), or extrinsic (e.g. drug paraphernalia, environments, people). Some previous studies suggest that the presence of, and
sensitivity to, cues is linked to greater addiction severity (Albertella et al., 2019; Antons et al., 2020; Jasinska et al., 2014; Zhang et al., 2020). Similarly, altered reactivity to gambling cues has been demonstrated in those with gambling disorder (Goudriaan et al., 2010; Limbrick-Oldfield et al., 2017). Further, these cues are capable of triggering relapse even well into abstinence and thus pose a challenge for recovery from addiction (Perry et al., 2014; Pitchers et al., 2018; Vafaie & Kober, 2022). This is thought to be the result of cues hijacking endogenous dopaminergic signaling, as over time dopamine is released in response to the cue rather than reward (Ljungberg et al., 1992; Yoshimi et al., 2011).

It is undisputed that previously neutral reward-paired cues can gain incentive salience following conditioning. However, there are individual differences in the manifestation of this behaviour. A common classification used in research on this phenomenon is classifying animals as goal-trackers or sign-trackers. Whereas goal-trackers direct their response towards the site of reward presentation, sign-trackers instead direct their responding towards reward-paired cues. Studies continually find that animals showing enhanced responsivity to reward-paired cues are more impulsive, make riskier decisions, and have impaired attention compared to their goal-tracking counterparts (Olshavsky et al., 2014; Swintosky et al., 2021). Likewise, sign-trackers seem to be more sensitive to reinforcing properties of drugs of abuse (Hilz et al., 2019; Meyer et al., 2012). Similarly, it has been shown that sign-trackers are willing to work harder for cocaine, are more resistant to extinction of drug-seeking, and have enhanced reinstatement of drug-seeking behaviour (Saunders & Robinson, 2011). The heightened addiction vulnerability demonstrated in sign-trackers vs goal-trackers may be due to differences in dopaminergic signaling during cue-reward learning. Indeed, sign-trackers have been shown to utilize
dopaminergic model-free learning via reward prediction error, where goal-trackers utilize non-
dopaminergic model-based learning (Gläscher et al., 2010; Schultz et al., 1997).

Furthermore, the addition of cues to behavioural tasks is known to induce decision-
making deficits. For example, it has been shown that the visual presentation of drug-paired cues prior to performing a delay discounting task was sufficient to induce discounting deficits in individuals with heroin addiction, when compared to being shown neutral cues (L. Yang et al., 2023). Similarly, the addition of cues to gambling tasks is also known to cause increased risky choice in humans and rodents alike (Barrus & Winstanley, 2016; Cherkasova et al., 2018). Casinos leverage this feature, as the salient audio-visual cues that accompany e.g. slot machine wins have been found to encourage pathological gambling and promote risky choice (Barrus & Winstanley, 2016; Cherkasova et al., 2018). Furthermore, the addition of reward concurrent cues to decision-making tasks has been shown to increase the ability of dopaminergic drugs to alter task performance in animal models (Barrus & Winstanley, 2016). Despite the plethora of research highlighting the maladaptive role of cues on both SUDs and behavioural addictions, further research is needed to address how these cues exert their effects over behaviour and if response to cues could be a target in the treatment of addiction.

1.5 What We Know from the Rat Gambling Task and Cocaine Self-Administration

The rat gambling task (rGT), loosely based on the IGT used clinically, incorporates complex schedules of reinforcement to closely model the decision-making processes involved in human gambling, and accounts for factors like cost-benefit calculations and uncertainty of outcomes. The rGT allows for the simultaneous assessment of decision-making strategy, impulsivity, motivation, and response latencies. In order to maximize their sugar pellet profits,
rats must develop a preference for the advantageous options associated with smaller per-trial gains but less frequent and lower time-out penalties and learn to avoid the “high-risk” options that yield larger wins per trial but also longer and more frequent punishments. Adding win-concurrent audiovisual cues to the rGT not only increases risky decision making, but also renders choice preferences insensitive to reinforcer devaluation, indicating that the decision process may be under habitual control in this task variant (Hathaway et al., 2021). This may explain why risky decision making persists throughout training on the cued rGT, and can be ameliorated by drugs which promote cognitive flexibility (Chernoff et al., 2021; Hathaway et al., 2021). The molecular mechanism underlying the effects of such habit-altering drugs on the rGT has yet to be investigated.

It has been found that risk preferring animals are uniquely vulnerable to the effects of cocaine self-administration, which further exacerbates risk-taking across rGT sessions (Ferland & Winstanley, 2017). This detrimental effect of cocaine use is not seen in rats with optimal decision-making strategies, indicating the existence of individual differences in response to drugs of abuse (Ferland & Winstanley, 2017). It is possible that risky choice and cue sensitivity act synergistically to confer addiction vulnerability. Indeed, it has been found that the addition of cues to the rGT facilitates risky decision-making, and that this maladaptive response is further exacerbated by the onset of cocaine self-administration (Ferland et al., 2019). Furthermore, our group has demonstrated that cue-induced increases in risky choice are mediated by dopamine release in the VTA and NAc of male rats (Hynes et al., 2020, 2021). We have further proven that inhibiting VTA dopamine neurons prevents cocaine-induced deficits in decision making on the cued rGT, while simultaneously causing increases in cocaine self-administration (Hynes et al.,
However, it is unknown whether other drugs of abuse such as opioids can cause similar decision-making impairments in this task.

1.6 Sex Differences

Failure to account for sex, or even to include females in studies, is a common shortcoming in animal research. Relevant to this dissertation, sex differences are known to arise in risk-based decision-making, and are perhaps owing to differential gonadal hormones and neural mechanisms that give rise to differences in cognition (Orsini, Brown, et al., 2022; Orsini, Truckenbrod, et al., 2022; Orsini & Setlow, 2017). The general consensus seems to be that females are more risk adverse than males (Harris & Jenkins, 2006; Ishii et al., 2018). To explain this, females (both humans and rats) have been shown to utilize different decision-making strategies than males, resulting in greater risk aversion and increased sensitivity to punishment (Ishii et al., 2018; Islas-Preciado et al., 2020; Liley et al., 2019; Orsini et al., 2016; Sarin & Wieland, 2016; Van Den Bos et al., 2012). Likewise, prominent sex differences are known to exist in the prevalence, onset, and severity of neuropsychiatric disorders, many of which implicate decision-making deficits in their core symptomology.

Sex differences are also apparent in addiction. Historically, more men than women are diagnosed with SUDs (Statistics Canada, 2015, 2017). However, differences in gender roles and access to drugs are thought to play a large role in this disparity. In support, the gender gap has been closing in recent years, as drug use and dependence becomes increasingly more common in males and females alike (Cotto et al., 2010; Holdcraft & Iacono, 2004; McHugh et al., 2018). Furthermore, it has been found that males and females have similar rates of drug use and likelihood to develop a SUD when access to drugs is controlled for (Anthony et al., 1994; Caris
et al., 2009; Van Etten & Anthony, 1999). Still, important sex differences in addiction behaviours are recognized, such as the fact that females cycle through the stages of addiction more quickly than males – a phenomenon known as “telescoping” – and are more prone to relapse (Bobzean et al., 2014; Haas & Peters, 2000; Maria et al., 2014). Similarly, animal studies indicate that females are quicker to both acquire and escalate drug-taking in cocaine self-administration paradigms (Hu et al., 2004; Larson et al., 2007; Lynch et al., 2001). This may be explained by females demonstrating increased sensitivity to the reinforcing properties of drugs of abuse (Harris & Jenkins, 2006; Liley et al., 2019; Orsini et al., 2016). Indeed, sex differences in response to cocaine have been shown to be mediated by an increased in VTA dopamine release, increased affinity of cocaine at dopamine transporters, and increased cue-reward associations in female mice, particularly during oestrus (Calipari et al., 2017). Furthermore, there is evidence to suggest that increased impulsivity predicts drug abuse to a greater extent in females compared to males, and that this lack of inhibitory control may mediate the faster progression of addiction seen in females (Weafer et al., 2015).

Sex differences in both decision-making and addiction-relevant behaviours may be governed in part by underlying differences in dopaminergic signaling. Indeed, females have been shown to have increased dopamine release in response to stimulants (Calipari et al., 2017; Manza et al., 2022). Furthermore, our group has previously demonstrated sexually dimorphic effects of dopamine inhibition on performance in the cued rGT. We found that the inhibition of VTA dopamine neurons reduced risky choice and motor impulsivity in males, while increasing risky choice in females (Hynes et al., 2021). Similarly, we have found that inhibiting dopamine release in the NAc caused a switch from risky to optimal decision-making in males, while reducing motor impulsivity yet leaving choice behaviour unaffected in females (Hynes et al., 2020). Thus,
sex remains an important factor to investigate in research concerning both decision-making and addiction.

1.7 Experimental Approach

Literature surrounding the etiology of addiction is vast, yet oftentimes contradictory. For example, there is evidence that addiction arises as a result of maladaptive habit formation, as well as evidence supporting the idea that addiction is instead a consequence of excessive goal-directed behaviour. Similarly, there are many seemingly opposing theories of addiction. Moreover, many researchers tend to consider different substance use disorders as equal. Yet, direct comparisons between different classes of drugs of abuse are few and far between, with studies that take biological sex into account being even fewer. The lack of synergy in addiction literature poses a barrier to the advancement of treatment options for this population, contributing to the fact that relapse rates remain incredibly high. The goal of the work presented in this dissertation is thus to further parse out the contribution of neurocognitive differences to addiction. To do so, we consider the influence of habit formation, salient audiovisual cues, and risky decision-making on addiction-relevant behaviour, as well as the effects of drug-taking on cognition, and assess whether these findings can be generalized across different drugs of abuse and biological sex.

**Experiment 1 (Chapter 3):** Evidence suggests that the formation of long-term memories is associated with increased levels of histone acetylation. Further, this makes such memories resistant to degradation, as evidenced by the persistence of habits, which may contribute to the formation and maintenance of addiction. In an attempt to pharmacologically induce habit formation via histone deacetylase inhibition, we administered sodium butyrate (NaBut) daily
following rGT testing in male rats. We hypothesized that NaBut would accelerate the development of risky option preferences during acquisition of the cued rGT, and that this would be accompanied by increased levels of histone acetylation in the striatum.

**Experiment 2 (Chapter 4):** Our group and others have repeatedly found cues to induce decision-making deficits. However, it was unclear if animals would willingly engage with the cued rGT over the uncued rGT, and how the same animal reacts in both the presence and absence of win-paired cues. We thus developed a novel behavioural task to gain insight into the individual differences that exist in this complex decision-making paradigm, and how volitional drug-taking impacts these cognitive processes in both males and females. We hypothesized that a subset of rats would prefer to engage in the cued task, and that these animals would display unique decision-making deficits and addiction vulnerability.

**Experiment 3 (Chapter 5):** To assess whether previous findings can translate across different drugs of abuse, we established a working model of fentanyl self-administration. Previous work from our lab showed that risk-preferring animals in the rGT were uniquely vulnerable to the effects of cocaine, and that cocaine self-administration further exacerbated preference for risk (Ferland et al., 2019; Ferland & Winstanley, 2017). We thus sought to assess the effect of fentanyl self-administration in the same paradigm, in both male and female rats. We hypothesized that fentanyl would similarly impair rGT performance, and that risk-preferring animals would be most affected.
Chapter 2: General Methods

2.1 Subjects

All animals used in these experiments were Long Evans rats, obtained from Charles River Laboratories (St Constant, Quebec), unless otherwise indicated. Subjects were pair- or trio-housed in Optirat® GenII cage system (Animal Care Systems Inc., Centennial, Colorado), lined with aspen chip bedding and were provided with paper towel and a plastic hut for enrichment. The housing room was maintained at 21 degrees Celsius on a 12 hour reverse light-dark cycle. All experiments took place during the dark cycle. Unless otherwise indicated, rats were fed a restricted diet of Laboratory Rodent Diet 2918 (LabDiet, St. Louis, Missouri), and were thus maintained at 85% of their free-feeding weight. Water was available ad libitum throughout the experiment. All testing and housing procedures were in accordance with the standards of 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

The rat gambling task took place in a bank of 16 standard five-hole operant chambers with a stimulus light and an infrared beam to measure nose pokes at the back of each hole (Med Associates, St. Albans, VT). Each chamber was housed within a ventilated sound-attenuating cabinet. On the opposite wall was a house-light and a food tray with a sucrose pellet dispenser attached (45 mg pellets, Bio-Serv, Flemington, NJ).

Self-administration took place in a separate bank of 16 standard Med Associates 5-hole operant chambers (Med Associates, St. Albans, VT) housed in ventilated sound-attenuating cabinets. Chambers were equipped with two retractable levers, two cue lights above the levers, a tone generator, an infusion pump, a centrally located food hopper, and a houselight. The operant
chambers were run according to MedPC programs authored by C.A.W. and controlled by an IBM-compatible computer.

2.3 The Cued and Uncued Rat Gambling Task

Food-restricted rats were first habituated to the operant chamber for 30 min. During habituation, 2 sugar pellets were placed in each aperture, and 10 were placed in the food magazine. Rats were then trained on a modified version of the five-choice serial-reaction time task in which rats were required to nose poke in one of four apertures, as indicated by illumination of hole 1, 2, 4, or 5 in a pseudo-random order. A nose poke within the illumination period resulted in the delivery of 1 sugar pellet. Each session lasted 30 min, or up to 100 trials – whichever occurred first. Subjects performed daily sessions until they met the criteria of ≥50 correct responses for three consecutive days. Following the completion of this training stage, the rats began the rat gambling task (rGT). Subjects initiated a rGT trial by making a nose poke in the illuminated food magazine tray. This caused the light to be extinguished and was followed by a 5 s intertrial interval (ITI). A nose poke during the ITI was recorded as a premature response and punished by a 5 s time-out in which no trials could be initiated. Following the ITI, apertures 1, 2, 4, and 5 were all illuminated for 10 s. Failure to respond during the 10 s illumination period was recorded as an omission and caused the food tray to illuminate, indicating the requirement for trial re-initiation via nose poke. A nose poke at one of the illuminated response holes during the 10 s illumination period was either rewarded with sugar pellets or punished with a time-out penalty as per the reinforcement schedule associated with that aperture (see Figure 2, P1–P4; 1–4 sugar pellets, 0.9–0.4 probability of reward, 5–40 s time-out duration).

Punished responses triggered the light inside the chosen aperture to flash at 0.5Hz throughout the time-out period, and any response made during this time had no programmed
consequences. Rewarded responses initiated the delivery of pellets in the food tray, and, in the
cued version of the task, were accompanied by concurrent 2 s tone/light cues. The complexity
and variability of these sensory cues scaled with reward size (single tone and illumination of
aperture for P1 cue, multiple tones and four patterns of flashing lights for P4 cue). The optimal
selection strategy was P2, due to its associated high delivery of reward and low probability,
infrequent punishments (0.2, 10 s). In contrast, selection of P3 and P4 was associated with a
disadvantageous selection profile due to more frequent and longer time-outs (0.5, 30 s; 0.6, 40 s)
resulting in fewer rewarded trials. The theoretical maximum number of pellets available per
session following exclusive choice of each option, assuming constant choice and collection
latencies, and no premature or omitted trials, were P1: 295; P2: 411; P3: 135; P4: 99. Side bias
was prevented by counterbalancing the P1–P4 aperture array such that half the subjects
performed version A or version B (left to right – A: P1, P4, P2, P3; B: P4, P1, P3, P2). To ensure
that rats had equal experience with all aperture contingencies, three forced choice sessions were
initially completed in which only a single aperture was illuminated pseudo-randomly during each
trial.

Training on the rGT continued for 5 sessions per week until statistically stable task
performance was reached. Behavioural stability indicates that behaviour is no longer changing
over time. Analysis of variance (ANOVA) confirmed stable performance for all variables across
the final 5 sessions (no significant effects of the within-subjects variable ‘session’).
Rats choose between 4 options, signaled by illumination of 4 holes, resulting in either delivery of reward or time-out penalties. Reinforcement contingencies are identical in both the cued and uncued task, but reward is accompanied by audiovisual cues that scale in complexity with size and magnitude of reward in the cued variant. Reward, time-out, and cue complexity vary with each P option.

### 2.4 Jugular Vein Catheter Implantation

Rats were implanted with indwelling right jugular vein catheters, constructed of Silastic silicone tubing (Dow Corning via VWR International, Edmonton, AB, Canada) and attached to back-mounted cannulae (Plastics One, Roanoke, VA, USA). Briefly, rats were anesthetized with isoflurane before two small incisions were made, one between the shoulder blades and the other on the right side of the chest. The backport was mounted between the shoulder blades and silicone tubing was directed over the right shoulder and slipped into the jugular vein. Following
surgery, animals were given 2 mg/kg Metacam diluted in saline for pain management and a topical hexachlorine ointment applied to both incision sites. Catheter patency was assessed by delivering 0.1 mL (I.V.) of a 10% ketamine HCL solution (Medisca Pharmaceuticals, British Columbia, Canada). Catheter patency was maintained with daily flushes of 0.2 mL catheter lock solution.

**2.5 Experimental Timelines**

For experiment 1, rats were gradually restricted to 85% of their free feeding weight before beginning nose-poke training. Once this behaviour was acquired, rats were randomly assigned to receive daily injection of NaBut or sterile water (vehicle; VEH), prior to beginning training and assessment on the cued rGT. Half of all animals were then terminated after 5 sessions of cued rGT to allow for the assessment of changes in histone acetylation at this timepoint. The other half of animals were run until behavioural stability, which was achieved in 25 sessions. On session 26, extinction testing was run. During this test, the cued rGT was run as usual, except sugar pellet delivery did not accompany any wins.

For experiment 2, rats were gradually restricted to 85% of their free feeding weight before beginning nose-poke training. Once this behaviour was acquired, rats received 2 sessions of lever training (1 session with the right lever, and 1 session with the left lever). After this, rats began training and assessment on both the uncued and cued rGT. Rats were first exposed to 5 sessions each of forced-lever forced-choice cued and uncued rGT, such that they could learn reward contingencies. Rats then received 15 sessions each of forced-lever free-choice cued and uncued rGT, on alternating days. After forced-lever free-choice training, animals were moved on to the choice rGT, whereby rats could freely choose which task (cued or uncued) and probabilistic outcomes they want to engage with on a trial-by-trial basis for 5 sessions per week.
until reaching stability, which occurred by session 24 for males and session 44 for females. Once
behavioural stability was achieved, all rats were implanted with jugular vein catheters. Rats were
assigned to receive cocaine or saline self-administration based on their performance in the rGT.
This was done to ensure no initial group differences existed. Following recovery, rats resumed
rGT testing and the first 5 post-surgery sessions were used as baseline data. On post-surgery
session 6, concurrent rGT and cocaine SA began. Rats were run in the rGT at the usual time and
then were returned to their home cage. Later in the evening, rats were subjected to 2 hrs of
ContAcc SA. Concurrent testing continued for a total of 10 sessions.

For experiment 3.1, all rats were implanted with jugular vein catheters before being
randomly assigned to self-administer fentanyl or saline. Rats were allowed a 1 week recovery
period before beginning self-administration. Rats underwent 10 sessions of ContAcc SA,
followed by 5 sessions of IntAcc SA. Following 15 total days of self administration, rats
underwent two weeks of forced abstinence in their home cage. Rats were tested for drug seeking
behaviour on days 1, 7, and 14 of forced abstinence.

For experiment 3.2, rats were gradually restricted to 85% of their free feeding weight
before beginning nose-poke training. Once this behaviour was acquired, rats began training and
assessment on the cued rGT. Once behavioural stability was achieved, which took 45 sessions,
all rats were implanted with jugular vein catheters. Rats were assigned to receive fentanyl or
saline self-administration based on their performance in the rGT. This was done to ensure no
initial group differences existed. Following recovery, rats resumed rGT testing and the first 5
post-surgery sessions were used as baseline data. On post-surgery session 6, concurrent rGT and
fentanyl SA began. Rats were run in the rGT at the usual time and then were returned to their
home cage. Later in the evening, rats were subjected to 2 hrs of ContAcc SA. Concurrent testing
continued for a total of 10 sessions. After 10 sessions of concurrent cued rGT and fentanylSA, rats underwent three weeks of forced abstinence in their home cage. Rats were assessed for drug-seeking behaviour on day 21 of forced abstinence. Daily cued rGT testing continued as normal during this time.

2.6 Statistical Analyses

Statistical analyses were performed using SPSS Statistics version 27.0 (IBM Corp, Armonk, NY, USA). The two key dependent variables of interest were score \([(P1 + P2) – (P3 + P4)]\), which provides an overall index of how optimal versus risky decision-making was during a given session, and percentage of premature responses (number of premature responses/total number of trials × 100). We also calculated and analyzed choice omissions (number of omissions/total number of trials × 100), number of trials completed, average choice latency, and average latency to collect a reward. The choice rGT had the additional variables of task preference, lever latency, and lever choice omissions. For self-administration data, the number of infusions obtained, number of responses on the active lever, and number of responses on the inactive lever were analyzed. Repeated measures analysis of variance (ANOVA) were performed using drug (NaBut/cocaine/fentanyl or VEH/saline) as a between subjects variable.

To meet normality assumptions, data were transformed for analyses as appropriate: rGT variables expressed as percentages were arcsine transformed. Untransformed data are presented for clarity. All data are expressed as mean ± Standard Error of the Mean (SEM). Differences were considered significant where \(p<0.05\); trend level differences where \(p\leq0.08\) are reported.
Chapter 3: Histone Deacetylase Inhibitor Sodium Butyrate Increases Risk-Taking During Acquisition of the Rat Gambling Task

3.1 Introduction

Habits, defined by their inflexibility or resistance to degradation and change, allow us to be more efficient in our day-to-day lives. However, habits can also be maladaptive. Substance use disorders and behavioural addictions may be considered in this regard. Changes in neural networks responsible for executive functioning and reward processing have been observed in these disorders, and behavioural studies support an association with maladaptive habit formation as well as deficits in goal-directed behaviour (Antons et al., 2020; Vandaele & Janak, 2018). Sensitivity to altered reinforcer value is commonly used to assess addiction-relevant behaviour in animal models, where it has been found that response for drug reinforcer is sensitive to devaluation after acute exposure, but insensitive following prolonged experience with that drug (López et al., 2016; Zapata et al., 2010). This may be reflective of a shift from goal-directed to habit-mediated processes with increased drug ingestion, and this may parallel the shift from recreational use to addiction.

One mechanism that may be critical for habit formation is histone acetylation, a form of epigenetic modification. Histone acetylation facilitates the transcription of DNA, resulting in increased gene expression. This process involves two different classes of enzymes: histone acetyltransferases (HATs) which acetylate histone tails and promote gene transcription, and histone deacetylases (HDACs) which remove acetyl groups and cause gene silencing (Kouzarides, 2007). After prominent behavioural events, HDACs are removed from gene promoter regions which in turn allows HATs to promote histone acetylation (Federman et al.,
Congruent to this, the production of long-term memories is associated with increased HAT and decreased HDAC activity (Gupta et al., 2010; Swank & Sweatt, 2001; Zuzina & Balaban, 2022). This gives rise to the transcriptional processes which support sustained functional neuronal changes and enable learning (Malvaez et al., 2018).

It is also known that memories formed as a result of HDAC inhibition can persist beyond the point at which normal memory fails, much like a habit (Stefanko et al., 2009). In support, researchers have found that HDAC inhibition accelerated habit formation during instrumental conditioning via increased histone acetylation in the dorsolateral striatum (Malvaez et al., 2018). Conversely, the overexpression of HDAC3 in the striatum prevented habit formation (Malvaez et al., 2018). Regarding the role of HDACs in addiction, changes in histone acetylation are evident following exposure to drugs of abuse and persist into withdrawal (Li et al., 2021; Renthal & Nestler, 2009). Thus, the persistent alterations in histone acetylation mirror the persistent nature of both habit formation and addiction.

HDAC inhibition is also capable of decreasing the threshold at which learning events lead to long-term memory formation (Stefanko et al., 2009). This may have implications in addiction, as learning about reward-paired cues may be enhanced. Indeed, HDAC inhibition is known to enhance cue-induced reinstatement of operant behaviour by strengthening the learned association between stimulus and reward (Ploense et al., 2013). Further, HDAC inhibition can facilitate the acquisition of morphine-induced conditioned place preference (Sanchis-Segura et al., 2009; Y. Wang et al., 2015). This premature entry of memories into stable long-term memory could then lead to inflexible, habitual behaviours that are maladaptive.

The rat gambling task (rGT), loosely based on the Iowa Gambling Task used clinically, uses complex schedules of reinforcement to closely model the decision-making processes
involved in human gambling, and accounts for factors like cost-benefit calculations and uncertainty of outcomes. The rGT allows for the simultaneous assessment of decision-making strategy, impulsivity, motivation, and response latencies. In order to maximize their sugar pellet profits, rats must develop a preference for the advantageous options associated with smaller per-trial gains but less frequent and lower time-out penalties, and avoid the “high-risk” options that yield larger wins per trial but also longer and more frequent punishments. Adding win-concurrent audiovisual cues to the rGT not only increases risky decision making, but also renders choice preferences insensitive to reinforcer devaluation, indicating that the decision process may be under habitual control (Hathaway et al., 2021). This may explain why risky decision making persists throughout training on the cued rGT, and can be ameliorated by drugs which promote cognitive flexibility (Chernoff et al., 2021; Hathaway et al., 2021). These habit-promoting effects of the cued version of the rGT complement observations that gambling speed and betting inflexibility, markers of habit formation, are increased with more experience in cued slot machine gambling (Ferrari et al., 2022).

We therefore hypothesized that HDAC inhibition would enhance risky choice during acquisition of the cued rGT by facilitating habitual control of behaviour. To test this hypothesis, we administered the non-specific class I HDAC inhibitor sodium butyrate (NaBut) after each cued rGT training session. We further hypothesized that administration of NaBut would cause an increase in histone acetylation in brain regions known to be involved in habit formation, and decrease sensitivity to reward omission.
3.2 Additional Methods

3.2.1 Subjects

Testing and housing procedures were in accordance with the standards of the Canadian Council of Animal Care, and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia. Subjects were 64 male Long Evans rats, obtained from Charles River Laboratories (St Constant, Quebec). Subjects were pair-housed in a housing room maintained at 21 degrees Celsius on a 12 hour reverse light-dark cycle. All experiments took place during the dark cycle. Rats were maintained at 85% of their free-feeding weight, and water was available ad libitum throughout the experiment.

3.2.2 Sodium Butyrate Preparation and Administration

Sodium butyrate (NaBut; product #303410 from Sigma-Aldrich, Oakville, ON, Canada) was dissolved in sterile water after the addition of 2N hydrochloric acid at 10% of the total solution volume. NaBut (1000 mg/kg I.P.) was administered at 2 mL/kg immediately following every forced-choice and free-choice rGT session.

3.2.3 Euthanasia and Tissue Collection

Rats tested until behavioural stability were anesthetized with isoflurane prior to carbon dioxide euthanasia. No tissue was collected from these animals.

The remaining rats were first anesthetized with isoflurane, then transcardinally opened and perfused with PBS. Brains were extracted immediately thereafter and placed in 4% paraformaldehyde for post-fixation and later sectioning and immunohistochemical analyses.

3.2.4 Immunofluorescence

Brains were post-fixed in 4% PFA, then cryoprotected with 30% sucrose in PBS and 0.01% sodium azide after which 35 μm-thick coronal sections were cut using a cryotome (Leica
Biosystems, Concord, ON). Immunohistochemistry comprised of staining for H3K27ac, a marker of histone acetylation. This stain followed an immunofluorescent protocol. Briefly, sections were washed in PBS then blocked with 0.3% Triton-X and 5% normal goat serum (NGS; Millipore Sigma S26-100ML) for 1 hr at room temperature (RT). Sections were incubated with primary antibody for H3K27ac (1:500 dilution; New England Biosystems 8173S) overnight at 4 °C. Sections were then incubated with Alexa-Fluor 488 secondary antibody (1:500 dilution; ThermoFisher Scientific A11034) and 4’6-diamidino-2-phenylindole (DAPI; 1:1000 dilution; Invitrogen P36935) for nuclear co-stain for 2 hr at RT for visualization using fluorescence microscopy. Sections were then washed in PBS, mounted on slides, and were later coverslipped with Krystalon.

All images were acquired using a Leica SP8 confocal microscope with a 63X objective and LasX software. Two randomly selected regions of interest (ROIs) of 489 x 496 μm were captured from each brain ROI – the dorsolateral and ventromedial striatum, orbitofrontal cortex, and prelimbic cortex. Image acquisition time was standardized to avoid saturation. Images were converted to simple segments with Ilastik software and cell counts of these images were performed using ImageJ software.

3.2.5 Computational Modelling

Reinforcement learning modelling was performed as described previously (Langdon et al., 2019), and briefly described below.

To analyze learning dynamics on the rGT, we modeled trial-by-trial choice preference with a series of reinforcement learning (RL) models (Sutton & Barto, 1998). Each of these models assumes choice preference on every trial probabilistically follows a latent Q-value for each option, which is iteratively updated on each trial according to the experienced outcome. To
focus model fitting on the evolution of choice preference during the learning phase, these models were fit to all valid choices on each trial from the first five free-choice sessions for all rats in each group.

In the simplest RL model (RL scaled punishment model), we assume that the equivalent punishment for a time-out interval on each negative-outcome trial scales linearly with the duration of the punishment. Our second model (RL scaled + offset punishment model) incorporates an additional offset to the linear transform between experienced time-out duration and the equivalent cost of that outcome on a given trial.

For both models, Q-values were initialized at zero for the first session, and we assumed Q-values at the start of a subsequent session (on the next day for example) were the same as at the end of the previous session (i.e., we modeled no inter-session effects on learning).

3.3 Results

3.3.1 Effect of NaBut on the Cued rGT

Acquisition

One rat was excluded from analyses due to failure to make a sufficient number of trials per session. During the first five cued rGT sessions, NaBut tended to increase risky decision-making, as indicated by a reduction in overall score (Figure 3; F1, 59 = 3.95, p = 0.052). This effect was not observed as training on the cued rGT progressed. This NaBut-induced reduction in score was driven by a significant increase in choice of the riskiest option, P4, during session 1-5 (Figure 4D; F1, 59 = 5.70, p = 0.02) combined with a trend towards reduction in choice of the most risk-averse option, P1 (Figure 4A; F1, 59 = 3.64, p = 0.061) compared to rats given VEH. Selection of P2 and P3 were unaltered by drug treatment (Figure 4B and 3C; all F’s ≤ 1.82, all p’s ≥ 0.189). These effects on decision-making were not evident in later training sessions. Score
distribution for both early acquisition (session 0-5) and after stability (session 20-25) are shown in Figure 5. We saw no differences between NaBut and VEH in latencies, omissions, or premature responding (descriptive statistics presented in Table 1; all F’s ≤ 2.19, all p’s ≥ 0.144) during any stage of cued rGT testing, indicating that NaBut does not induce psychomotor slowing, affect motivation, or increase motor impulsivity.

![Graph](image_url)

**Figure 3. Effect of NaBut on cued rGT score.**

There was a trend for NaBut to increase risky decision-making during acquisition of the cued rGT, as evidenced by a reduction in overall score during sessions 1-5 (week 1; F1, 59 = 3.95, p = 0.052). This effect was not observed as training on the cued rGT progressed. Data are presented as mean ± between-subjects SEM.
Figure 4. Effect of NaBut on P Choices.

NaBut-induced reduction in score during cued rGT acquisition was driven by changes in percent choice of the most optimal and most risky options: P1 and P4, respectively. A Rats administered NaBut showed a trend towards a reduction in P1 choice (F1, 59 = 3.64, p = 0.061) compared to rats given VEH. B&C Percent choice of P2 and P3 were not significantly affected by drug treatment. D NaBut-treated rats chose P4 significantly more than VEH-treated rats during acquisition of the cued rGT (F1, 59 = 5.70, p = 0.02). Data are presented as mean ± between-subjects SEM.
Figure 5. Score distribution.

Score distribution on the cued rGT for both A: early acquisition (session 0-5; n = 64) and B: after stability (session 20-25; n = 32).

Table 1. Descriptive statistics for additional rGT variables.

Descriptive statistics (mean ± SEM) for additional rGT variables by drug treatment group (VEH or NaBut) during sessions 1-5 of cued rGT testing.

<table>
<thead>
<tr>
<th></th>
<th>Choice latency (s)</th>
<th>Collection latency (s)</th>
<th>Omissions (#)</th>
<th>Prematures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEH</td>
<td>1.88 ± 0.06</td>
<td>1.57 ± 0.14</td>
<td>2.30 ± 0.20</td>
<td>27.39 ± 0.80</td>
</tr>
<tr>
<td>NaBut</td>
<td>1.92 ± 0.06</td>
<td>1.13 ± 0.05</td>
<td>2.61 ± 0.29</td>
<td>27.60 ± 0.81</td>
</tr>
</tbody>
</table>

**Extinction**

When tested in extinction, all rats took longer to make a choice (session: F_{1,27} = 37.31, p < 0.001) and to approach the food hopper following a win (session: F_{1,27} = 45.35, p < 0.001).

This was seen regardless of whether animals were treated with NaBut or VEH (choice latency –
drug: $F_{1,27} = 0.30$, $p = 0.586$; collection latency – drug: $F_{1,27} = 0.004$, $p = 0.950$). However, these effects were significantly less pronounced in risk-prefering rats (choice latency – session*risk preference: $F_{1,27} = 13.21$, $p = 0.001$; collection latency – session*risk preference: $F_{1,27} = 8.414$, $p = 0.007$; Figure 6). All rats also made more omissions during extinction (session: $F_{1,27} = 5.97$, $p = 0.021$) regardless of drug treatment (drug: $F_{1,27} = 0.04$, $p = 0.836$), but premature responding was not altered (session: $F_{1,27} = 2.23$, $p = 0.157$). Likewise, all rats completed fewer trials during extinction (session: $F_{1,27} = 42.11$, $p < 0.001$), regardless of drug (drug: $F_{1,27} = 0.69$, $p = 0.412$). This was particularly evident in optimal decision-makers (trials completed – session*risk preference: $F_{1,27} = 17.21$, $p < 0.001$). While extinction did not affect overall score (session: $F_{1,27} = 0.02$, $p = 0.897$), rats nevertheless increased choice of P1 and decreased choice of P2, effects that were again largely driven by optimal decision-makers (P1 – session: $F_{1,27} = 21.29$, $p < 0.001$; session*risk preference: $F_{1,27} = 6.08$, $p = 0.02$; P2 – session: $F_{1,27} = 13.23$, $p = 0.001$; session*risk preference: $F_{1,27} = 4.01$, $p = 0.055$). All animals increased their choice of P4 (session: $F_{1,27} = 4.01$, $p = 0.055$). As shown in Figure 7, none of the changes in choice were dependent on drug treatment (session*drug, session*drug*risk preference: all $Fs < 1.46$; $p > 0.238$).
Figure 6. Response latencies during extinction.

A When tested in extinction, all rats took longer to initiate trials (F1,27 = 37.31, p < 0.001), regardless of drug treatment. There was a significant choice latency*risk preference (F1,27 = 13.21, p = 0.001) interaction, whereby risk-preferring animals were quicker to initiate trials than their optimal counterparts. B Rats also took longer to approach the food hopper following a win during extinction (F1,27 = 45.35, p < 0.001). There was a significant collection latency*risk preference (F1,27 = 8.414, p = 0.007) interaction, whereby risk-preferring animals were quicker to collect reward than their optimal counterparts. Data are presented as mean ± between-subjects SEM.
Figure 7. P choices during extinction.
Extinction altered which P options rats were engaging with. A Rats chose P1 significantly more during extinction (F1,27 = 21.29, p < 0.001), regardless of drug treatment. A significant P1*risk preference interaction (F1,27 = 6.08, p = 0.02) reveals that this effect is driven by optimal rats choosing P1 more during extinction. B Rats chose P2 significantly less during extinction (F1,27 = 13.23, p = 0.001) regardless of drug treatment. There was a trend-level P2*risk preference interaction (F1,27 = 4.01, p = 0.055), indicating that risky animals continued to choose P2 during extinction. C Choice of P3 was unaffected by drug treatment and risk preference during extinction. D Rats chose P4 significantly more during extinction (F1,27 = 8.20, p = 0.008), regardless of drug treatment. Data are presented as mean ± between-subjects SEM.

3.3.2 Effect of NaBut Treatment on ex vivo Marker of Histone Acetylation
Systemic HDAC inhibition should globally decrease histone deacetylation by HDACs and thus increase histone acetylation in areas where HATs are active. We thus expected to see increased levels of histone acetylation in brain regions active during our operant task. As the dorsolateral striatum is known to play an important role in habit formation (Malvaez et al.,
2018), we were primarily interested in confirming histone acetylation in this brain region. Using immunofluorescence, we found significantly higher levels of H3K27Ac, a marker of histone acetylation, in the ventromedial striatum (t = -2.26, p = 0.025) of NaBut-treated rats one hour following the last operant session and drug delivery, relative to VEH-treated rats. We also found a trend for higher levels of H3K27ac in the dorsolateral striatum (t = -1.58, p = 0.075) of NaBut-treated rats, relative to VEH-treated rats. Similarly, there was a trend for higher cell counts in the prelimbic cortex (t = -1.70, p = 0.055) of NaBut-treated rats, relative to VEH-treated rats. Conversely, NaBut-treatment did not impact levels of H3K27ac in the orbitofrontal cortex (t = -1.10, p = 0.145). Descriptive statistics for cell counts are presented in Table 2, graphical representation of cell counts are presented in Figure 8, and representative images shown in Figure 9.

Table 2. Descriptive statistics for cells counts in striatal (DLS, VMS) and frontal (OFC, PrL) subregions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Drug</th>
<th>N</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLS</td>
<td>VEH</td>
<td>6</td>
<td>198.20</td>
<td>7.11</td>
</tr>
<tr>
<td></td>
<td>NaBut</td>
<td>5</td>
<td>218.20</td>
<td>11.02</td>
</tr>
<tr>
<td>VMS</td>
<td>VEH</td>
<td>6</td>
<td>194.25</td>
<td>15.24</td>
</tr>
<tr>
<td></td>
<td>NaBut</td>
<td>5</td>
<td>260.30</td>
<td>26.39</td>
</tr>
<tr>
<td>OFC</td>
<td>VEH</td>
<td>8</td>
<td>76.75</td>
<td>15.16</td>
</tr>
<tr>
<td></td>
<td>NaBut</td>
<td>8</td>
<td>98.50</td>
<td>12.73</td>
</tr>
<tr>
<td>PrL</td>
<td>VEH</td>
<td>8</td>
<td>97.88</td>
<td>14.00</td>
</tr>
<tr>
<td></td>
<td>NaBut</td>
<td>8</td>
<td>140.62</td>
<td>20.82</td>
</tr>
</tbody>
</table>
Figure 8. Graphical representation of cell counts in striatal (DLS, VMS) and frontal (OFC, PrL) subregions.

A Cell counts were significantly higher in the ventromedial striatum ($t = -2.26, p = 0.025$) of NaBut-treated rats one hour following the last operant session and drug delivery, relative to VEH-treated rats. There was also a trend for higher cell counts in the dorsolateral striatum ($t = -1.58, p = 0.075$) of NaBut-treated rats, relative to VEH-treated rats. B There was a trend for higher cell counts in the prelimbic cortex ($t = -1.70, p = 0.055$) of NaBut-treated rats, relative to VEH-treated rats. Data are presented as mean ± between-subjects SEM.
Figure 9. Representative 63X images from NaBut or VEH-treated rats.

We found significantly higher levels of H3K27Ac, a marker of histone acetylation, in the ventromedial striatum (t = -2.26, p = 0.025) of NaBut-treated rats one hour following the last operant session and drug delivery, relative to VEH-treated rats. There was also a trend for higher cell counts in both the dorsolateral striatum (t = -1.58, p = 0.075) and the prelimbic cortex (t = -1.70, p = 0.055) of NaBut-treated rats, relative to VEH-treated rats. Cell counts did not differ by drug-treatment in the orbitofrontal cortex.

3.3.3 Effect of NaBut Treatment on Reinforcement Learning Parameters

Group-level mean posterior estimates for each parameter were compared between the NaBut and VEH groups. Generally speaking, results across both models followed similar patterns. In both models, the NaBut-treated group exhibited a higher positive learning rate than the control group. Additionally, estimates for the negative learning rate in the NaBut-treated group were lower than controls. Overall, this indicates that HDAC inhibition resulted in enhanced learning from winning outcomes, and reduced impact of the time-out penalties on choice patterns, ultimately leading to higher preference for the risky options. The experimental
group also exhibited lower beta estimates, indicating that choice patterns for these rats did not follow the stored values for each option as closely as in the VEH-treated animals. This could indicate that HDAC inhibition induced more exploratory behaviour, or otherwise introduced more noise into the decision-making process. Model fits are shown in Figure 10.

![Figure 10. Reinforcement learning model fits.](image)

Reinforcement learning model fits: basic (top) and scaled + offset (bottom). Across both models, the NaBut-treated group exhibited a higher positive learning rate (eta positive) than the control group. Additionally, estimates for the negative learning rate (eta negative) in the NaBut-treated group were lower than controls. NaBut-treated rats also exhibited lower beta estimates, indicating that choice patterns did not follow the stored values for each option as closely as in the VEH-treated animals.

### 3.4 Discussion

In keeping with our hypotheses, we found that NaBut accelerated the development of a preference for the risky options during acquisition of the cued rGT. Computational modeling of the first 5 cued rGT sessions revealed that NaBut-treated rats had a higher eta positive and lower eta negative than the VEH-treated controls, indicating greater learning from wins but a reduced
impact of losses. On balance, wins therefore had a greater influence on decision making during task acquisition when NaBut was administered after each training session. Ex vivo immunohistochemical analyses confirmed that levels of histone acetylation were higher in ventral and dorsal striatum, as well as prelimbic cortex, in NaBut-treated rats at the end of session 5 of drug treatment, when the behavioural effect was maximal. Although risk-preferring rats were less sensitive to the effects of reward omission on response latencies and choice preferences, perhaps indicative of weaker goal-directed control, NaBut treatment did not alter this effect.

Despite increasing risky choice during task acquisition, we did not observe impairments in other aspects of task performance as a result of treatment with NaBut. Motivation to perform the task, as indicated by levels of omissions, trials completed, or response latencies, were all unaffected by the drug. Similarly, we saw no changes in levels of motor impulsivity. These findings are somewhat in contrast with other groups that have observed changes in motivation for a variety of reinforcers after HDAC inhibition. However, NaBut-induced histone acetylation will only augment the expression of genes that are actively being transcribed at the time of administration. As such, its effects are both time and activity dependent, as well as region-specific (Elvir et al., 2019; Schroeder et al., 2008). For example, treatment with an HDAC inhibitor 30 minutes prior to testing facilitated ethanol self-administration, whereas treatment 3 hours prior to testing reduced ethanol self-administration (Elvir et al., 2019; Jeanblanc et al., 2015; Qiang et al., 2015). This pattern of results presumably reflects functional distinctions in the genes subject to regulation by HDACs and HATs at different time-points. Our experimental design was modeled on a previous study in which administration of NaBut after each behavioural training session enhanced habitual control of behaviour, in the absence of motivational changes.
(Malvaez et al. 2018). The specificity of this time-locked effect likely reflects the function of the genes active in the post-training period, as compared to those active during the training session itself. As such, administration of NaBut prior to initiation of each daily test-session may result in a very different pattern of results.

HDAC inhibition facilitates the acquisition of both cocaine- and morphine-induced CPP (Raybuck et al., 2013; Y. Wang et al., 2015). However, low dose HDAC inhibition also accelerates the extinction of this behaviour (Raybuck et al., 2013; Y. Wang et al., 2015). This points to the role of HDACs in learning, as rats both acquired and extinguished behaviour faster under the influence of HDAC inhibition. This, together with findings from the reinforcement learning models employed in the current study, points to enhanced learning, specifically from saliently cued wins, as an explanation for why NaBut-treated rats in our study made more risky decisions during the first five sessions of cued rGT acquisition.

However, this effect was transient, as these animals did not stabilize at a higher level of risk taking. Assuming NaBut altered learning through enhancing memory formation, the time-limited nature of the drug’s effect may indicate that memory consolidation only influences the development of a decision-making strategy within the first five sessions. The time period in which NaBut treatment altered behaviour matches the phase of the task when learning rates are highest, as animals are essentially deciphering the reinforcement contingencies associated with each option through trial and error. Beyond this time frame, it is possible that the internal model of the task and the reinforcement contingencies associated with each option are largely in place. If so, then memory for task events would theoretically have less effect on subsequent performance, which may explain why the NaBut-treated group does not continue to become progressively riskier. Alternatively, by amplifying memory consolidation, HDAC inhibition may
reduce the number of sessions required for the task model to form. Whereas this is largely in place within five sessions in NaBut-treated rats, control animals may instead develop an understanding of the task structure more slowly due to a more labile consolidation period.

Both of these interpretations imply that NaBut treatment simply accelerates the learning process, without necessarily altering it. Given that the overall bias towards the risky options is similar by the end of training, this would arguably be a reasonable conclusion, were it not for the insights obtained from the reinforcement learning models. Previous applications of these algorithms to data from the uncued rGT demonstrate that risky decision making arises from reduced learning from the punishing time-out periods (Langdon et al. 2019). The addition of reward-paired cues to the task exacerbated this effect, at both the individual and group levels (Hathaway et al., 2022; Langdon et al., 2019). While win-paired cues facilitate the development of a bias toward the risky options, the computational data suggest they do not fundamentally alter the way in which this bias forms. In contrast, although NaBut administration similarly decreased learning from the penalties, it also concomitantly increased learning from rewards. As such, decreasing HDAC function did not just speed up the inevitable development of a preference for the risky options, but actually altered the way in which that strategy was learned.

This conclusion resonates with previous work exploring how HDAC inhibition potentiates memory formation. HDAC inhibitors can result in memory for an event that would otherwise not be recalled while facilitating stimulus-response associations (Ploense et al., 2013; Stefanko et al., 2009), and result in more long-lasting memories than those formed under normal conditions. Such data have led researchers to question whether HDAC inhibitors should be viewed as more than simple memory enhancers that boost the natural process of memory formation. Research from auditory cue-reward training suggests that systemic administration of
an HDAC class III inhibitor selectively potentiates responding to auditory cues that are both reward-predictive and reward-concurrent, and expanded the tonotopic areas in primary auditory cortex encoding those select frequencies while also tightening the specific tuning curves (Bieszczad et al., 2015). As such, the authors concluded that HDACs may regulate “informational capture”, which reflects the transformation of a sensory experience into a perceptually vivid long-term memory, through control of plasticity in sensory cortices. HDAC inhibition therefore results in stronger memories for events that are richer in sensory content.

Given that the rewards were heavily cued, this theory could also explain why NaBut resulted in enhancement of memory for rewarding events at the expense of memory for the negative consequences of a choice. Hence, rats significantly preferred P4 which is associated with the highest potential reward, most complex and variable cue, but the longest and most frequent penalty. Similarly, NaBut tended to decrease choice of P1, the option paired with the most frequent yet smallest reward and least salient cue. If the richness of the sensory percept is critical for driving the effects of NaBut on decision-making patterns, then we would predict a null or lesser effect if the cues were removed from the task, or if the relationship between cue complexity and reward size was inverted.

While this account may seem plausible, this interpretation does not account for the lower levels of beta- the inverse temperature term- seen in NaBut treated animals. This suggests that drug-treated animals are not adhering to the latent Q-values as strongly when making their choices, but responding in a more exploratory fashion. This low level of beta could represent compensation for a more rapidly adopted, yet less accurate, model, which may explain why the score for NaBut-treated rats is ultimately similar to that of control-treated animals by the end of training. Alternatively, this could also reflect elements of model-free learning, in that animals
may be more responsive to trial-by-trial feedback rather than acting based on the model they have developed, leading to noisier decision-making patterns (Daw & Tobler, 2014).

The processes underlying model-free learning are thought to overlap with habit formation, although these concepts are somewhat distinct (Gillan et al., 2015). In parallel to effects on learning, habit formation is increased by HDAC inhibition (Malvaez et al., 2018). Habit formation causes behavioural inflexibility and thus behaviour does not change following motivational loss (Gardner & Rebar, 2019). This may explain why NaBut-treated animals were more insensitive to the effects of the time-out penalties in our study. Similar decision-making strategies are seen in individuals with SUDs, who tend to place more weight on rewards and less on punishments that may accompany such rewards (Chen et al., 2020; Gowin et al., 2013).

The standard test for the presence of habitual control is determining that changing the reward value, typically through satiation or pairing with an aversive experience, does not alter behaviour. We have already demonstrated that choice patterns in the cued rGT are insensitive to such reinforcer devaluation (Hathaway et al., 2021). We therefore chose to test animals in extinction, with the prediction that NaBut-treated rats would be less sensitive to reward omission. While score was not altered by extinction testing, choice of P1 and P4 increased at the expense of P2. These effects are similar to those observed with the uncued rGT, and suggest decision making on the cued rGT is not insensitive to the cancellation of reward delivery. Changes in selection of P1 and P2 were less apparent in risk-preferring rats, as were the expected increases in choice and collection latency when reward was omitted, suggesting choice in these animals is less goal-directed than that of optimal decision-makers. However, we did not observe any differential effects in NaBut-treated rats. Previous reports show that NaBut can impair extinction of cocaine-induced CPP when extinction sessions are followed by NaBut injections.
(Raybuck et al., 2013). We may therefore have observed a decreased rate of extinction had we adopted a similar experimental design. Combined with the transient effects of NaBut treatment on choice during task acquisition, these results suggest that the effects of HDAC inhibition during task acquisition may initially accelerate the development of risky choice, but have limited effects once animals have settled on a decision-making strategy.

Given that NaBut-treated rats responded similarly to control animals when tested in extinction, do we have any evidence that the increase in risky choice we observed early in training was due to the dominance of habitual over goal-directed behavioural control? A pattern of increased histone acetylation in brain regions synonymous with the regulation of habit formation, such as the DLS and OFC (e.g. Gremel & Costa, 2013), in animals euthanized at the time the behavioural effect was observed would have supported this hypothesis. However, while we observed a trend-level increase in H3K27ac levels in the DLS, the most pronounced changes in histone acetylation were within the VMS and PrL, areas instead associated with goal-directed behaviour (Balleine & Dickinson, 1998; Burton et al., 2015). As noted above, computational modelling of the data from NaBut-treated rats suggest the HDAC inhibitor had enhanced risky choice not only by amplifying the latent cognitive mechanism responsible for such decision making in untreated rats, namely decreased learning from the punishing time-outs, but also by amplifying learning from rewards. It is tempting to speculate that the latter is driven by increased transcriptional activity in the PrL and VMS, whereas the former could arise through the DLS. Certainly, both the PrL and VMS have been extensively implicated in allowing cue-reward associations to guide behaviour. Future studies in which HDAC inhibitors are infused directly into these regions after each training session could test this hypothesis, and help determine the roles played by these different areas in regulating decision making.
In conclusion, the current study demonstrates that enhancing learning through administration of HDAC inhibitors can facilitate risky decision making on the cued rGT, and further indicate that the behaviour of risk-preferring rats is less goal-directed than optimal decision-makers on this task. However, whether HDAC inhibition drives risky choice through enhanced habitual control of behaviour is unclear. Altered levels of histone acetylation have been observed in some, but not all, rats following social defeat and early life stress, as well as following exposure drugs of abuse (Albuquerque Filho et al., 2017; Hollis et al., 2011; Li et al., 2021; Renthal & Nestler, 2009). Further, it has been found that chronic, but not acute, exposure to cocaine and stress decreases HDAC5 gene expression resulting in increased histone acetylation (Renthal et al., 2007). This presents a potential mechanism underlying why some individuals may progress to addiction while many do not, despite being exposed in a similar fashion. Exploring the divergent mechanisms through which addictive behaviour can develop will hopefully expand the range of therapeutic options available for individuals with addiction disorders.
Chapter 4: Examining the Interaction Between Gambling, Drug-Taking, and Decision-Making Using a Novel Behavioural Task

4.1 Introduction

The prevalence of problem gambling in 2018 was 0.6% of the Canadian population, but these rates tend to be much higher in individuals with comorbid psychiatric disorders (McIntyre et al., 2007; Williams et al., 2021). For example, the prevalence of problem gambling is much higher in those with co-occurring substance use disorders (SUDs), where it has been reported that up to 60% of problem gamblers meet the diagnostic criteria for a SUD (Barnes et al., 2015; Lorains et al., 2011; Rush et al., 2008). This could be explained by shared risk factors, such as increased impulsivity and impaired decision-making. Despite very high comorbidity rates between neuropsychiatric disorders, concurrent disorder models are underutilized in preclinical research.

Problem gamblers, as well as individuals with SUDs, are known to value immediate, short-term rewards over adopting the more optimal strategy to maximize long-term reward (Ciccarelli et al., 2019; Moses et al., 2020; L. Yang et al., 2023). Similarly, these individuals also show greater levels of risky decision-making (Chen et al., 2020; Stevens et al., 2013; G. Wang et al., 2013). Indeed, Stout and colleagues found male drug abusers were found to make less optimal decisions in a laboratory-based gambling task, driven by greater importance attributed to wins and hyposensitivity to punishment, and have found cocaine abuse to be correlated with motivational and decision-making deficits, but not learning and memory impairments (Stout et al., 2004, 2005). Results from female participants were less clear in these studies. Similarly, work from our lab has shown that risk-prefering male rats trained on a cued gambling task are
hyposensitive to punishment (Langdon et al., 2019). Females were not included in this dataset. These findings emphasize the multidimensional relationship between neuropsychiatric disorders, as well as the need to investigate the role of sex on interactions between drug abuse and decision-making.

The rat gambling task (rGT) is translationally valuable, as it incorporates complex schedules of reinforcement to closely model human gambling, while accounting for factors like cost-benefit calculations and uncertainty of outcomes. The rGT also allows for the simultaneous measurement of decision-making, response latencies, and impulsivity, making it an ideal task for detecting sensitivity to psychiatric disorders. While the majority of animals adopt an optimal decision-making profile in order to maximize reward, a subset of animals trained on the rGT exhibit risky decision-making. Moreover, when trained on a cued variation of the rGT, in which rewards are accompanied with flashy lights and jingles akin to those at casinos, the subset of risk-preferring rats is larger than on the uncued rGT (Barrus & Winstanley, 2016; Ferland et al., 2019). We have also shown that cocaine-taking exacerbates risk-taking on the rGT, and this is particularly true of rats trained on the cued rGT (Ferland et al., 2019; Ferland & Winstanley, 2017). Computational modeling indicates this cue-induced increase in risky choice is driven by greater insensitivity to punishment (Langdon et al., 2019), much like the decision-making bias observed in those with psychiatric disorders. It has also been found that training on the cued rGT decreases basal levels of dopamine in the nucleus accumbens, which may be responsible for driving the increase in risky decision-making and cocaine-taking observed in these rats (Ferland et al., 2019).

Experiments to date have never used rGT task variation (i.e. cued or uncued) as a within-subjects variable. We have therefore developed a novel behavioural task, dubbed the choice rGT,
in order to determine if preference for cues could be associated with increased impulsivity and addiction-relevant behaviours. We found that rats have unique risk and task preferences, which determines how they respond in the task. Findings indicate that while the presence of salient audiovisual cues is preferred to their absence, these cues are sufficient to induce decision-making biases on the same rats within the same session, underscoring the risk to human gamblers since audiovisual cues often accompany gambling environments. Future experiments with this novel behavioural task will allow us to more realistically model the multifaceted impairments seen in human psychiatric disorders.

4.2 Additional Methods

4.2.1 Subjects

Testing and housing procedures were in accordance with the standards of the Canadian Council of Animal Care, and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia. The subjects of this experiment consisted of 32 male and 32 female Long Evans rats, obtained from Charles River Laboratories (St Constant, Quebec). Subjects were pair-housed in a housing room maintained at 21 degrees Celsius on a 12 hour reverse light-dark cycle. All experiments took place during the dark cycle. Rats were maintained at 85% of their free-feeding weight, and water was available ad libitum throughout the experiment.

4.2.2 Choice rGT

Once the criteria for nosepoke training was met (as described in general methods), all rats underwent two sessions of lever training, during which one lever press corresponded to a one sugar pellet reward. Following the completion of these training stages, the rats began a modified version of the rat gambling task (rGT), depicted in Figure 11. Rats were first exposed to 5
sessions each of forced-lever forced-choice cued and uncued rGT, such that they could learn reward contingencies. Rats then received 15 sessions each of forced-lever free-choice cued and uncued rGT, on alternating days. The subject initiated a trial by making a left or right lever press, followed by a nose poke in the illuminated food magazine tray. One lever was required for initiating cued rGT trials, whereas the opposite lever was required for initiating uncued rGT trials. Which lever was paired with which rGT version was counterbalanced between subjects. After forced-lever free-choice training, animals were moved on to the choice rGT, whereby rats could freely choose which task (cued or uncued) and probabilistic outcomes they want to engage with on a trial-by-trial basis for 5 sessions per week until reaching stability. Analysis of variance (ANOVA) confirmed stable baseline performance for all variables across the final 5 sessions (no significant effects of the within-subjects variable ‘session’). All male rats reached stability by session 24, and females by session 44.
Figure 11. Schematic of the choice rGT operant paradigm.

In the choice rGT, rats first make a lever press to choose between the cued and uncued task variants. Rats then choose between 4 P options, signaled by illumination of 4 holes, resulting in the delivery of either reward or time-out penalty. Reward magnitude, time-out penalty, and cue complexity (if the cued variant was selected) vary with each different P option, as depicted here.

4.2.3 Cocaine Preparation and Self-Administration

Catheter lock solution was made by dissolving heparin in glycerol and saline to make a 10% heparin solution, which prevents blood from clotting and compromising catheter patency. Next, 500 units of gentamicin was added to the catheter lock solution to reduce the possibility of infection.

Cocaine hydrochloride (Medisca, St. Laurent, Quebec) was dissolved in sterile saline at a concentration of 6 mg/mL and administered at a dose of 0.5 mg/kg per infusion.
4.2.4 Concurrent Choice rGT and Cocaine Self-Administration

Rats were allowed 1 week recovery from catheterization surgery before proceeding with behavioural testing. After recovery, rats were run on the choice rGT during the morning, as before. After 5 baseline choice rGT sessions, self-administration sessions commenced in the evening, wherein rats were placed in operant chambers with cocaine (0.5 mg/kg/infusion), or saline available on a fixed ratio-1 (FR-1) schedule for 2 hrs. At the start of every session, rats received one infusion to cue drug availability. During drug delivery, the active lever had an illuminated cue light above it. Lever presses to the active lever resulted in a 4.5 s infusion of cocaine (or saline), during which the cue light flashed. Presses to the inactive lever were recorded but had no programmed consequences. A 10 s time out period followed each infusion, during which the cue light above the active lever was not illuminated, and active lever presses had no consequences. Following the time out, the cue light was re-illuminated to signal cocaine availability. There was no limit placed on maximum number of infusions per session. Rats were subjected to a 0.1 mL (I.V.) dose of 10% ketamine solution to test for patency of catheters on the first and last day of self-administration. Flushing with saline and catheter lock solution occurred everyday of testing.

This paradigm was devised in order to determine to what extent task preference (cued or uncued) influences drug taking, and conversely how drug-taking influences decision making on 10 concurrent choice rGT sessions.

4.2.5 Computational Modeling

The diffusion model was fit to behavioural data from the choice rGT using fast-dm-30.2 (Voss et al., 2010, 2015; Voss & Voss, 2007, 2008). Lever choice, corresponding to a choice for either an uncued or cued trial, and response times (RTs) for these choices were input to the
model. Figure 12 shows the task structure, and which behavioral measures from the task were used for modeling. Fast-dm calculates predictive cumulative distribution functions (CDFs) for choices and RTs, and then uses a partial differentiation equation solver to model the evolution of the probability distribution forward in time. Parameters are optimised by using an implementation of the Nelder-Mead method (Nelder & Mead, 1965). Further details about diffusion modelling using fast-dm can be found in (Voss et al., 2015). Multiple models were tested using different combinations of parameters that were fit to all trials where a lever choice for an uncued or cued trial was made to identify the parameter combination that produced best model fits. Validation of this best fitting model was carried out on behavioural data from the final three stable acquisition sessions for all rats (n = 64). Data from individual rats were modeled separately. As carried out previously (Aylward et al., 2020; Hales et al., 2016), and following recommendations given in (Voss et al., 2015), model fit was assessed using Kolmogorov-Smirnov (KS) test statistics output by fast-dm-30.2. The KS test statistic is the maximum absolute vertical distance between the empirical and the predicted CDFs of the RT distributions. For multiple trials in a task, n, it is computed as:

$$KS = \max_{i=1\ldots n} |eCDF(RT_i) - pCDF(RT_i)|,$$

where eCDF and pCDF are the empirical and predicted CDFs, respectively, and RTi is the response latency in trial i. p-values < 0.05 indicate that the model does not demonstrate a good fit. Subjects with model fit KS statistics p < 0.05 were excluded from analyses. Table 5 details the number of animals removed and the reasons for exclusion. The parameter combination that produced the best model fit was selected and used to model the behavioural data. This best fitting model had six parameters: starting point (zr), boundary separation (a), drift rate (v), non-decision RT (t0), the difference in speed of response execution between the two responses (d), and the
variability in the starting point (szr). The other parameters that can be fit within fast-dm-30.2: variability in drift rate (sv), variability in non-decision RT (st0) and percentage of contaminants (p); were set to 0. For the first analysis all six parameters (zr, a, v, t0, d and szr) were fit to all trials within a session (all trials condition). For the choice analysis, all parameters except d and szr were fit to trials split by whether the rat proceeded to make an optimal choice (defined as either a P1 or P2 choice) or a risky choice (a P3 or P4 choice). The outcome analysis was the same, except for trials were split by whether the rat went on to make a valid P choice in any hole, or made a premature response. For both the choice and outcome conditions, trials for the three consecutive stable baseline sessions were combined for each individual rat to allow sufficient trial numbers for model fitting. In the model, the upper boundary represents a decision for a cued trial, while the lower boundary represents a decision for an uncued trial.

Figure 12. Schematic of the drift diffusion model.

The drift diffusion model was applied to lever choice data (final 3 sessions of acquisition) using fast-dm-30.26. zr, v, a, and t0 were fit to all data, trial data split by whether the rat next made a choice or premature response, and trial data split by subsequent P-choice (P1/P2 or P3/P4).
4.2.6 Statistical Analyses

The two key dependent variables of interest were score \([ (P1 + P2) - (P3 + P4) ] \), which provides an overall index of how optimal versus risky decision-making was during a given session, and percentage of premature responses (number of premature responses/total number of trials × 100). We also calculated and analyzed choice and lever omissions (number of omissions/total number of trials × 100), number of trials completed, percent choice of the cued lever (task choice), average latency to initiate a lever press, average latency to choose an option, and average latency to collect a reward.

As per previous analysis of rGT data, rats were classified as risk-preferring or optimal decision-makers, depending on whether the average score was below or above zero. This classification was done based on all trials in a choice rGT session, as well as using just cued or uncued trials for comparison. Rats were also divided into three distinct groups based on their task choice. Cued-preferring rats were defined as those which selected the cued rGT on ≥ 60 percent of trials, whereas uncued-preferring rats engaged with the cued rGT on ≤ 40 percent of trials. Those with a neutral preference chose the cued rGT between 40 and 60 percent of the time.

Repeated measures analysis of variance (ANOVA) were therefore performed using within-subject variables of task chosen (2 levels: cued, uncued), and between subjects variables of sex, risk preference (2 levels: risk-preferring, optimal), and task preference (3 levels: cued-preferring, neutral, uncued-preferring). Task preference analyses were only conducted in males, as very few females could be categorized as uncued-preferring. To meet normality assumptions, data were transformed for analyses as appropriate: Choice rGT variables expressed as percentages were arcsine transformed. Untransformed data are presented for clarity. All data are
expressed as mean ± Standard Error of the Mean (SEM). Differences were considered significant when \( p < 0.05 \); trend level differences when \( p \leq 0.08 \) are reported.

Modelling data were analyzed as follows. For the all trials condition, two-way ANOVAs with sex (male or female) and risk status (optimal or risky) as between-subjects factors were used to analyze model parameters. Mixed ANOVAs with choice/outcome (optimal P1/P2 vs risky P3/P4 choice or valid choice vs premature response) as the within-subjects factor, sex (male or female), and risk status as between-subjects factors were used for the choice and outcome analyses. Following significant main effects or interactions, one-way ANOVAs, paired t-tests or independent samples t-tests were performed as post-hoc tests as appropriate. Huynh-Feldt corrections were used to adjust for violations of the sphericity assumption, and Bonferroni correction was applied for multiple pairwise comparisons. Statistics are reported with the ANOVA F-value (degrees of freedom, error) and p-value as well as any post-hoc p-values.

All statistical analysis was conducted using SPSS 28.0.0.0 for Windows (IBM SPSS Statistics), and all graphs were produced using GraphPad Prism 9.4.0 for Windows (Graphpad Software, USA).
4.3 Results

4.3.1 Choice rGT

4.3.1.1 Task Preference

Just over half of each group preferred to perform cued rGT trials, and this was comparable across sex (Table 3). However, whereas the remaining male rats were relatively evenly split between neutral and uncued-preferring, only two females preferred the uncued task. As such, task preference could only be used as a between-subjects factor in analyses of data from males.

Table 3. Task and risk preference by sex in the choice rGT.

<table>
<thead>
<tr>
<th>SEX</th>
<th>TASK PREFERENCE</th>
<th>RISK PREFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUED</td>
<td>UNCUED</td>
</tr>
<tr>
<td>MALES</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>FEMALES</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>11</td>
</tr>
</tbody>
</table>

4.3.1.2 Task Choice

Sex had no effect on percentage of cued trials selected over time ($F_{1,60} = 1.628, p = 0.207$). Although risk preference similarly had no overall effect on the percentage of time in which the cued task was chosen in a given session (risk preference: $F_{1,60} = 1.017, p = 0.317$), risk-preferring rats exhibited a steady and progressive increase in choice of the cued rGT vs uncued rGT as training on the choice rGT continued (session*risk preference: $F_{4,240} = 4.474, p = \ldots$
0.002). As such, risky choice was associated with progressively greater preference for the cued rGT. This is depicted in Figure 13.

![Figure 13. Percent choice of the cued lever by risk preference.](image)

Data are presented as mean ± between-subjects SEM.

### 4.3.1.3 Score

All rats can be classified as either risky or optimal, based on their average score (main effect of risk preference on score: \( F_{1,60} = 251.792, p = 0.000 \)). The proportion of rats classified as risky vs optimal does not tend to differ by sex. We have previously demonstrated that the proportion of risky rats is larger when rats are trained on the cued vs uncued rGT. The magnitude of this effect was smaller in the choice rGT. We did however note that rats which were classified as risk-preferring using data from cued trials were also risk-preferring on the uncued task, and optimal decision-makers were likewise classified as such regardless of whether cued or uncued trials were used to calculate score (Table 4). For the remainder of these analyses, classification of animals as risky or optimal was therefore based on all trials in the final 5 choice rGT sessions.
We have previously seen that rats trained on the cued rGT have significantly lower scores, indicative of greater risky choice, than those trained on the uncued task. Similarly, when calculated separately from cued and uncued trials within the same sessions, rats obtained a lower score on cued vs uncued trials (task chosen: $F_{1,60} = 4.560, p = 0.037$; Figure 14A). Score distribution is shown in Figure 15.

Score did not differ between males and females (sex: $F_{1,60} = 0.014, p = 0.907$). However, there was a trend-level sex*risk preference interaction ($F_{1,60} = 3.327, p = 0.073$). We also observed significant task chosen*sex*risk preference ($F_{1,60} = 6.512, p = 0.013$) and task chosen*session*sex*risk preference interactions ($F_{4,240} = 2.516, p = 0.042$), indicating that risk-prefering males showed the steepest decrease in score over time on the cued rGT. This is depicted in Figure 14B&C.

We saw no effect of task-preference on score: cued-prefering males showed statistically similar degrees of risky choice as uncued-prefering males (task preference: $F_{2,29} = 0.484, p = 0.621$; Figure 16).

Table 4. Risk preference in the choice rGT by task choice and sex.

<table>
<thead>
<tr>
<th>SEX</th>
<th>RISK PREFERENCE</th>
<th>TASK</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>CUED</td>
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<td>MALES</td>
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<tr>
<td></td>
<td>OPTIMAL</td>
<td>17</td>
</tr>
</tbody>
</table>
Figure 14. Score by task choice on the choice rGT.

A Score by task (pooled sex). Score is shown by task choice and risk preference for B males, and C females. Data are presented as mean ± between-subjects SEM.
Figure 15. Score distribution for choice rGT.

Score distribution for choice rGT, separated by task, A: cued and B: uncued trials (n = 64).

Figure 16. Score by task choice and task preference on the choice rGT.

Data is shown for A males and B females. Data are presented as mean ± between-subjects SEM.

4.3.1.4 Premature Responses

Overall, rats made significantly more premature responses when engaging with the cued rGT ($F_{1,60} = 51.783$, $p = 0.000$; Figure 17A). However, this was critically influenced by task preference, as depicted in Figure 17C&D (task preference: $F_{2,26} = 2.819$, $p = 0.078$; task
chosen*task preference interaction: F_{2,26} = 17.718, p < 0.001), such that cued-preferring animals made more premature responses but only when performing the cued rGT. Similarly, uncued-preferring rats made more premature responses when performing the uncued rGT. In other words, male rats made more premature responses when engaging with their preferred task.

As depicted in Figure 17B, risk-preferring rats also made more premature responses, regardless of task chosen or task preference (risk preference: F_{1,60} = 6.878, p = 0.011; risk preference x task chosen: F_{1,60} = 0.708, p = 0.403; risk preference x task preference: F_{2,26} = 2.197, p = 0.131). Sex did not affect levels of premature responding (F_{1,60} = 0.581, p = 0.449).

**Figure 17. Percentage of premature responses by task choice on the choice rGT.**

A Percent premature by task (pooled sex). B Percent premature by task choice and risk preference. Percent premature is shown by task choice and task preference for C males, and D females. Data are presented as mean ± between-subjects SEM.
4.3.1.5  Response Latencies

4.3.1.5.1  Lever Choice Latency

Rats were consistently slower to respond on the lever that triggered a cued rGT trial than on the corresponding uncued rGT lever (task chosen: F<sub>1,60</sub> = 43.984, p = 0.000; Figure 18A), and this effect held regardless of task preference or risk preference (task chosen x task preference: F<sub>2,29</sub> = 2.457, p = 0.103; task chosen x risk preference: F<sub>1,60</sub> = 0.028, p = 0.868). However, cued-prefering rats were faster to initiate trials, regardless of which option was chosen (task preference: F<sub>2,29</sub> = 8.163, p = 0.002). Similarly, risk-prefering rats also made this choice more rapidly (risk preference: F<sub>1,60</sub> = 21.435, p = 0.000). Sex did not influence lever choice latency (sex: F<sub>1,60</sub> = 1.941, p = 0.169).

4.3.1.5.2  Choice Latency

Neither the task chosen nor task preference impacted the latency to choose between the four options (all F’s < 2.769, p’s > 0.101). However, both risk-prefering rats and females were quicker to choose between the four options (risk preference: F<sub>1,60</sub> = 4.668, p = 0.035; sex: F<sub>1,60</sub> = 4.814, p = 0.032).

4.3.1.5.3  Reward Collection Latency

Collection latency was significantly reduced during cued rGT trials when compared to uncued rGT trials made by the same rats (F<sub>1,60</sub> = 35.618, p = 0.000; Figure 18B), indicating animals were quicker to collect reward when it was cued. Likewise, cued-prefering male rats were significantly faster to collect reward than neutral or uncued-prefering rats (F<sub>1,29</sub> = 8.889, p = 0.001). Collection latency was not significantly affected by risk preference or sex (all F’s < 0.571, all p’s > 0.453).
Data is shown for A lever and B collection latency. Data are presented as mean ± between-subjects SEM.

4.3.1.6 Omissions

There was no significant main effect of task preference on lever choice omissions ($F_{1,29} = 2.105, p = 0.140$). However, optimal decision-makers made more lever choice omissions than their risk-preferring counterparts (risk preference: $F_{1,60} = 4.700, p = 0.034$), and females omitted more trials than males (sex: $F_{1,60} = 6.808, p = 0.011$). We also found a significant session*sex interaction ($F_{4,240} = 3.44, p = 0.009$), with females making more lever choice omissions over time.

Once the rGT trial had been initiated, choice omissions were low, regardless of which task was chosen (task chosen: $F_{1,60} = 0.612, p = 0.437$). However, cued-preferring animals made significantly fewer omissions than neutral- and uncued-preferring animals (task preference: $F_{2,26} = 4.488, p = 0.021$), an effect that was most pronounced on uncued trials (task chosen*task preference interaction ($F_{2,26} = 11.128, p < 0.001$). Risk-preferring rats also made fewer omissions than optimal decision-makers ($F_{1,60} = 10.195, p = 0.002$). Sex did not affect omission rates ($F_{1,60} = 0.314, p = 0.578$).
4.3.1.7 Trials Completed

Task preference did not impact the number of trials completed ($F_{2,29} = 0.414, p = 0.665$). However, consistent with data from the rGT, risk-preferring rats completed fewer trials than optimal decision-makers ($F_{1,60} = 69.557, p < 0.001$). Risk-preferring rats incur more frequent and lengthy time-out periods as a consequence of selecting P3 and P4 more frequently, during which they are unable to initiate trials, hence the lower trial counts. Females also tended to complete more trials ($F_{1,60} = 2.714, p = 0.059$), even though the proportion of risk-preferring animals was similar across sex (see Table 3).

4.3.2 Computational Modeling

Descriptive statistics for rGT behavioural data used for modeling is presented in Table 5. In the baseline all trials analysis, there were no main effects of sex or risk preference on starting point, boundary, drift rate, or non-decision time (all $F$’s $< 1.628$, all p’$s > 0.200$). Likewise, there were no interactions with session, sex, or risk preference for starting point, boundary, or drift rate (all $F$’s $< 2.923$, all p’$s > 0.092$). As shown in Figure 19, there was a main effect of sex ($F_{1,60} = 6.385, p = 0.014$) and risk preference ($F_{1,60} = 6.028, p = 0.017$) on non-decision time, whereby both males and risk-preferring rats independently had shorter non-decision times when it comes to initiating a trial via lever press. Session was not significant ($F_{2,120} = 0.052, p = 0.949$).

In the baseline P choice analysis, there were no main effects or interactions with P choice, sex, or risk preference for starting point or boundary (all $F$’s $< 0.942$, all p’$s > 0.339$). There was however a main effect of P choice on drift rate (Figure 20; $F_{1,41} = 4.137, p = 0.044$), indicating that drift rates were more positive (towards cued decision boundary) for risky choices compared to optimal choices. There was also a choice*sex*risk preference interaction ($F_{1,39} =$
5.109, p = 0.029). Post-hoc tests revealed that in male rats, drift rates were neutral for optimal rats making optimal choices, but for risky choices, drift rates were positive (indicating stronger evidence for a cued choice). In male risky rats, drift rates were similarly positive for both optimal and risky choices. There was no difference between drift rates for optimal or risky choices in optimal or risky female rats. For non-decision time we found a main effect of P choice (Figure 19; F_{1,39} = 14.758, p < 0.001), whereby overall non-decision times were longer for risky choices, and risk preference (F_{1,39} = 23.273, p < 0.001), whereby optimal rats have longer non-decision times than risky rats. However, there were also significant sex*risk preference (F_{1,39} = 5.856, p = 0.020) and P choice*sex (F_{1,39} = 5.891, p = 0.020), and P choice*risk preference interactions (F_{1,39} = 22.319, p < 0.001), indicating that the main effects are driven specifically by optimal rats having longer non-decision times when they make a “non-preferred” risky P choice, with this being more pronounced in male optimal rats compared to female optimal rats.

In the baseline outcome analysis there were no main effects or interactions with outcome, sex, or risk preference for boundary or drift rate (all F’s < 2.494, all p’s > 0.120). There was a trend level effect of outcome on starting point (F_{1,49} = 3.594, p = 0.064), suggesting that decision starting points for trials followed by a premature response were not biased towards the cued boundary (unlike for decisions followed by a P choice). There were no main effects or interactions with sex or risk preference (all F’s < 1.769, all p’s > 0.189). There was however a main effect of both outcome (Figure 22A; F_{1,49} = 25.824, p < 0.001) and risk preference (Figure 22B; F_{1,49} = 12.232, p < 0.001) on non-decision time, whereby overall non-decision times were longer on trials followed by a premature response, and longer in optimal rats. There was also a significant outcome*risk preference interaction (F_{1,49} = 5.778, p = 0.020). Post-hoc tests indicate that for trials followed by a P choice or premature response, risky rats have shorter non-decision
times than optimal rats, and non-decision times are especially long for optimal rats on trials followed by a premature response.

Table 5. Descriptive statistics for rGT behavioral data used for computational modeling.

<table>
<thead>
<tr>
<th>ANALYSIS</th>
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<td></td>
<td>32</td>
<td>F</td>
<td>7(^{\text{v}})</td>
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</tr>
<tr>
<td>OUTCOME</td>
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<td>M</td>
<td>9(^{\text{c}})</td>
<td>53</td>
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<td></td>
<td>32</td>
<td>F</td>
<td>2(^{\text{c}})</td>
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\(^{\text{v}}\)Excluded because rat did not perform sufficient trials of each choice type (>10 trials) to allow for model fitting

\(^{\text{c}}\)Excluded because poor model fit based on KS statistic (p<0.05).
Figure 19. Non-decision times for baseline all trials analysis.

Analysis of baseline all trials analysis reveals a main effect of sex ($F_{1,60} = 6.385, p = 0.014$) and risk preference ($F_{1,60} = 6.028, p = 0.017$) on non-decision time.
Figure 20. Drift rate for P choice by sex and risk preference on the choice rGT.

There was a main effect of choice on drift rate ($F_{1,41} = 4.137, p = 0.044$), as well as a P choice*sex*risk preference interaction ($F_{1,39} = 5.109, p = 0.029$).

Figure 21. Non-decision time for P choice by sex and risk preference on the choice rGT.

For non-decision time we found a main effect of P choice ($F_{1,39} = 14.758, p < 0.001$) and risk preference ($F_{1,39} = 23.273, p < 0.001$). There were also significant sex*risk preference ($F_{1,39} = 5.856, p = 0.020$) and P choice*sex ($F_{1,39} = 5.891, p = 0.020$), and P choice*risk preference interactions ($F_{1,39} = 22.319, p < 0.001$).
There was a main effect of both outcome (Figure 17A; F1,49 = 25.824, p < 0.001) and risk preference (Figure 17B; F1,49 = 12.232, p < 0.001) on non-decision time, as well as a significant outcome*risk preference interaction (F1,49 = 5.778, p = 0.020).

### 4.3.3 Cocaine Self-Administration

As expected, there was a significant main effect of drug on infusions taken (F1,37 = 11.218, p = 0.002), but no effect on active or inactive lever presses (all F’s < 1.286, all p’s > 0.264). Likewise, we found no significant main effect of sex, risk preference, or task preference on infusions of cocaine taken (all F’s < 0.563, all p’s > 0.576).

### 4.3.4 Concurrent Choice rGT and Cocaine Self-Administration

There was no effect of drug on task preference, score, choice latency, collection latency, lever latency, premature responses, or trials completed (all F’s < 0.413, all p’s > 0.524). There was however a significant main effect of drug on P choice omissions and lever choice omissions (F1,45 = 5.352, p =0.025 and F1,47 = 7.716, p = 0.008, respectively), whereby cocaine-taking animals made more omissions. Omission data is depicted in Figure 23.
Figure 23. Omissions by drug on concurrent choice rGT sessions.

Session 1-5 represent post-surgery baseline data. Data is presented for both A lever choice omissions and B P choice omissions. Data are presented as mean ± between-subjects SEM.

4.4 Discussion

Previous data from our lab indicates that rats trained the cued rGT have a more risky decision-making profile in addition to increased motor impulsivity than rats trained on the uncued rGT (Barrus & Winstanley, 2016; Ferland et al., 2019; Hathaway et al., 2022). This is corroborated by current findings, which further indicate that this effect is observed in a within-subjects design whereby rats can choose between the cued and uncued rGT on a trial-by-trial basis. Thus, we can infer that cues are capable of inducing decision-making and impulse control deficits that are situation-dependent, and that these deficits do not generalize outside of the task.

Our findings that uncued-preferring males are slower to collect reward, are slower to initiate trials, and make more P choice omissions while having no differences in score from their cued-preferring counterparts may indicate that this subset of animals is less motivated to engage in the task. This is supported by the modeling results, where male optimal rats had weaker evidence accumulation for optimal choices and longer non-decision times before making risky
choices. Similarly, our finding that optimal rats are slower to initiate trials and make more omissions than their risk-preferring counterparts suggests that these rats may be less engaged in the task. This may suggest a neural overlap in requirements for uncued task preference, optimal decision-making, and motivational demand. For example, dopamine signaling is known to be involved in both decision-making and motivational control (Bromberg-Martin et al., 2010; Salamone et al., 2018; Soutschek et al., 2023), and may thus be altered in these subsets of animals. Alternatively, dopamine signaling may be altered in risky and cued-preferring animals. This may be a more plausible explanation for the observed differences, as dopamine agonists have been previously shown to induce risky decision-making, particularly under the influence of cues (Hirschbichler et al., 2022; Mortazavi et al., 2023; Simon et al., 2011), which may point to aberrant dopamine signaling as the cause for behavioural differences observed in risky and cued-preferring rats in our study.

The results from our computational modelling do indeed provide support for the interpretation that rats with an optimal decision-making profile are less engaged in the task than those with a preference for the risky options. Non-decision time, the main parameter that differs between optimal and risky rats, corresponds to components of the total choice latency that are unrelated to the evidence accumulation process, i.e. for encoding and/or response preparation or other extraneous factors. Optimal rats have longer non-decision times on the task, and this is particularly apparent on choices that are followed by a premature response, or when optimal rats are making a “non-preferred” risky choice. One interpretation is that the longer non-decision times in these rats could represent distraction, indicative of non-optimal and premature choices being the result of a deviation from their preferred strategy. This supports the idea that optimal rats are less engaged in the task, but the modeling more precisely narrows this down to being
specifically driven by performance on a subset of trials – those where optimal rats are deviating from optimal choices.

Our finding that the cues reduce latency to collect reward is supported by previous work from our lab comparing performance on the cued vs uncued rGT (Ferland et al., 2019). The ventral pallidum plays an important role in reward-seeking, particularly in the presence of cues (Richard et al., 2018). It has been found that activity in the ventral pallidum is associated with how quickly animals collect reward following a cue under both instrumental and Pavlovian conditioning (Richard et al., 2018). Furthermore, ventral pallidal neurons have been shown to fire in response to auditory cues that predict sucrose reward, as well as following even subconscious exposure to drug-paired cues (Childress et al., 2008; Tindell et al., 2004). This may suggest that the ventral pallidum is activated to a greater extent when animals are engaging in the cued rGT, as opposed to the uncued rGT. The ventral pallidum may also encode hedonic value (Tindell et al., 2006), so our finding that rats prefer the cued over uncued rGT further supports the role of the ventral pallidum driving the behavioural effects seen on the cued rGT.

Our finding that rats are slower to initiate cued trials may be indicative of a post-reward slowing and altered dopaminergic signaling. In support, psychomotor slowing following slot machine wins is evident in research in both human gamblers and rodents (Ferrari et al., 2022; Peters et al., 2010; J. Schreiber & Dixon, 2001). Psychomotor slowing is also known to predict reinforcement learning deficits, but not altered reward sensitivity, in individuals with a history of major depression, and may thus indicate vulnerability to depression (Letkiewicz et al., 2022). Our findings may thus be indicative of dopaminergic dysregulation, as dopamine signaling is implicated in psychomotor control, reward-based reinforcement learning, and cue-guided decision-making (Daw & Tobler, 2014; Martinot et al., 2001; M. Yang et al., 2022). Indeed, our
group has previously shown that animals trained on the cued rGT are hypodopaminergic at baseline while having increased sensitivity to reward (Ferland et al., 2019). In further support, disrupting dopamine signaling during reward delivery has been shown to slow responding on subsequent trials (Fischbach & Janak, 2019). Thus, it may be the case that cues simultaneously reduce basal dopamine, resulting in increased latency to initiate trials, while increasing reward-induced dopamine efflux, resulting in reduced latency to collect reward. Furthermore, this altered dopaminergic signaling may leave animals more vulnerable to developing neuropsychiatric disorders such as depression.

The null effects of cocaine-taking on decision-making and impulsivity in the present study was unexpected, as previous research from our lab suggests that cocaine-taking increases risk-taking and impulsivity on the rGT (Ferland et al., 2019; Ferland & Winstanley, 2017). Current findings may be due to differences in the task itself, such as the incorporation of a lever choice to initiate trials in the choice rGT. Indeed, it could be that the incorporation of a lever choice allows the dopamine response from the previous trial to dissipate prior to subsequent P choice selection. In contrast, a new trial is initiated via nosepoke during collection of sugar pellet reward in our classic cued and uncued rGT paradigms. Alternatively, cocaine-induced decision-making deficits between past and current studies could be the result of different training paradigms. Particularly, rats were trained on both the cued and uncued rGT prior to beginning free-choice choice rGT, as opposed to only being trained on e.g. the cued rGT in prior experiments. However, this explanation is less likely as we have seen cocaine-induced decision-making deficits following training on exclusively the cued (Ferland et al., 2019), as well as exclusively the uncued (Ferland & Winstanley, 2017) rGT. However, our finding that cocaine-taking animals made more omissions in the choice rGT is congruent with findings that cocaine
use causes motivational deficits. Indeed, motivational deficits are evident in individuals with cocaine use disorder (Moreno-López et al., 2017). Moreover, SUDs are highly comorbid with major depression, a disorder characterized by profound motivational deficits (Conway et al., 2006; Quello et al., 2005). It has also been shown that cocaine self-administration causes increased omissions and response latencies on the 5-choice serial reaction time task in animal models, indicative of motivational deficits (Dalley et al., 2005). Further preclinical findings indicate that rats previously exposed to cocaine have reduced motivation to avoid aversive outcomes (Nguyen et al., 2015, 2018). This reduced motivation may explain why it is increasingly difficult to break out of the cycle of addiction with increased drug use, and may implicit drug use having a causal role in the etiology or exacerbation of depressive symptoms.

Overall, our current findings highlight the detrimental effect of heavily cued environments on decision-making, as well as the risk of motivational deficits arising as a consequence of cocaine use. In future experiments, the use of this novel behavioural task will allow us to more realistically model the multifaceted impairments seen in human psychiatric disorders.
Chapter 5: Increased Risky Choice During Forced Withdrawal From Fentanyl on the Cued Rat Gambling Task

5.1 Introduction

Addiction is a widespread epidemic, with over 20% of Canadians meeting criteria for substance use disorder (SUD) in their lifetime (Statistics Canada, 2015). The opioid crisis continues to surge, resulting in an average of 21 deaths per day due to opioid toxicity in Canada alone (Public Health Agency of Canada, 2023). Moreover, 81% of reported opioid toxicity deaths in 2023 involved fentanyl, highlighting the fact that fentanyl is rapidly replacing heroin in the opioid abuse landscape and thus the need to further study the effects of this particular drug (Belzak & Halverson, 2018; Government of Canada, 2023).

High comorbidity with other neuropsychiatric disorders such as gambling disorder (GD) is also evident in individuals with SUDs (Chen et al., 2020). Neurocognitive deficits are present in many such disorders and may thus represent a shared risk factor in the etiology of both SUDs and other neuropsychiatric conditions. Indeed, individuals with SUDs and GD often have impaired decision-making, which negatively impacts multiple life domains (Brand et al., 2005; Chen et al., 2020; Krmpotich et al., 2015). These decision-making impairments also correlate with treatment failure and addiction severity (Baldacchino et al., 2012; Fishbein et al., 2005). In support, a risky decision-making profile in the Iowa gambling task (IGT) has been seen in individuals with a dependence on various drugs of abuse including marijuana, cocaine, and opioids, and is known to predict relapse to such substances (Bolla et al., 2003; Lemenager et al., 2011; Stevens et al., 2013). Interestingly, individuals who meet diagnostic criteria for both SUD and GD have exacerbated decision-making impairments when compared to those who meet the
diagnostic criteria for either condition alone (Krmpotich et al., 2015). Conversely, longer periods of abstinence in methamphetamine-dependent individuals are associated with improvement in decision-making strategies on the IGT (G. Wang et al., 2013). However, it remains unclear whether these decision-making deficits are a cause or consequence of drug use due to various environmental and situational factors that are difficult to control for in human research.

The cued rat gambling task (rGT) is a translationally valuable task based on the IGT, which incorporates salient audiovisual cues and complex schedules of reinforcement to closely model human gambling while accounting for factors like cost-benefit calculations and uncertainty of outcomes. Thus, the cued rGT can be used to reliably assess whether decision-making deficits precede, or are a result of, drug use. Previous research from our lab has shown that risk preferring animals are uniquely vulnerable to the detrimental effects of cocaine self-administration, which further exacerbates risk-taking across rGT sessions (Ferland & Winstanley, 2017). This detrimental effect of cocaine use is not seen in rats with optimal decision-making strategies, indicating the existence of individual differences in response to drugs of abuse (Ferland & Winstanley, 2017). Furthermore, the addition of cues to the rGT facilitates risky decision-making, and that this maladaptive response is further exacerbated by the onset of cocaine self-administration (Ferland et al., 2019). It is possible that risky choice and cue sensitivity thus act synergistically to confer addiction vulnerability.

The goal of the current experiment was therefore to assess whether exposure to fentanyl self-administration would result in the same decision-making impairments on the cued rGT as have previously been observed in response to cocaine. We first report successful implementation of the fentanyl self-administration procedure in both male and female Long Evans rats in
Experiment 1, followed by the combination of fentanyl self-administration in animals trained on the cued rGT in Experiment 2.

5.2 Additional Methods

5.2.1 Subjects

Testing and housing procedures were in accordance with the standards of the Canadian Council of Animal Care, and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia. Subjects were peer housed and the housing room was maintained at 21 degrees Celsius on a 12 hour reverse light-dark cycle. All experiments took place during the dark cycle.

Experiment 1

Subjects were bred in-house from wildtype Long-Evans (Charles River, St. Constant, QC) females and males that expressed Cre recombinase (Cre) in neurons producing tyrosine hydroxylase (TH) (Long-Evans-TG(TH-Cre)3.1Deis, RRRC #00659; Rat Resource and Research Centre, RRRC, Columbia, MO). Only animals not expressing Cre (transgene negative; TG-), and therefore unsusceptible to the transduction of the Cre-dependent vector, were used in this experiment. Subjects consisted of 29 rats (14 male, 15 female). Food and water were available ad libitum throughout the experiment.

Experiment 2

Subjects consisted of male and female Long Evans rats (N=48; 24 male, 24 female), obtained from Charles River Laboratories (St Constant, Quebec). Rats were maintained at 85% of their free-feeding weight. Water was available ad libitum throughout the experiment.
5.2.2 Behavioural Testing

Experiment 1

A timeline of events for Experiment 1 is presented in Figure 24A. Experimentally naïve rats were allowed 1 week recovery from catheterization surgery before proceeding with behavioural testing. Rats were first subjected to 10 2 hour sessions of continuous access self-administration (ContAcc SA), wherein they were placed in operant chambers with fentanyl (1.5 mcg/kg/infusion), or saline available on a fixed ratio-1 (FR-1) schedule. At the start of every session, rats received one infusion to cue drug availability. Active lever presses resulted in a 5 s infusion of fentanyl (or saline), during which the cue light flashed. A 20 s time out period followed each infusion in which the cue light above the active lever was not illuminated, and active lever presses had no consequences. Following the time out, the cue light was re-illuminated to signal drug availability. Inactive lever presses had no programmed consequences. Following 10 sessions of ContAcc SA, rats received 5 2 hour sessions of intermittent access (IntAcc) SA, wherein rats were only able to engage in drug-taking for 5 min every 30 min.

There was no limit placed on the maximum number of fentanyl infusions per session. Rats were subjected to a 0.1 mL (I.V.) dose of 10% ketamine solution to test for patency of catheters on days 1, 10, and 15 of self-administration.

Following 15 total days of self-administration, rats underwent two weeks of forced abstinence in their home cage. Rats were tested for drug seeking behaviour on days 1, 7, and 14 of forced abstinence. On these sessions, rats were placed in the operant chambers for one hour sessions, during which active lever presses resulted in illumination of cue light but no drug delivery, and inactive lever presses had no programmed consequences. After the last behavioural test session, animals were euthanized via live decapitation. Immediately following
decapitation, trunk blood was collected and stored on ice. Brains and hearts were immediately extracted and flash frozen on dry ice. These tissues were donated to another researcher.

Experiment 2

A timeline of events for Experiment 2 is presented in Figure 24B. Rats were trained to stability on the cued rGT, as described above, before undergoing jugular vein catheterization. Following recovery, rats resumed rGT testing and the first 5 post-surgery sessions were used as baseline data. On post-surgery session 6, concurrent cued rGT and fentanyl SA began. Rats were run in the rGT at the usual time and then were returned to their home cage. Later in the evening, rats were subjected to 2 hrs of ContAcc self-administration of 1.5 mcg/kg/inf fentanyl or saline as described above. Concurrent testing continued for a total of 10 sessions.

After 10 sessions of concurrent cued rGT and fentanyl SA, rats underwent three weeks of forced abstinence in their home cage. Rats were tested for drug seeking behaviour on day 21 of forced abstinence. Daily cued rGT testing continued as normal during this time. Animals were humanely euthanized following completion of the last behavioural test session.
5.2.3 **Fentanyl Preparation**

Fentanyl citrate (product #119228 from CDMV, St-Hyacinthe, QC, Canada) was diluted in sterile saline and administered at a dose of 1.5 mcg/kg per infusion.

5.3 **Results**

**Experiment 1**

As expected, rats allowed to self-administer fentanyl obtained more infusions and made more active lever presses than their saline self-administering counterparts (see Figure 25). There was a significant main effect of drug on infusions obtained over the last 5 sessions of ContAcc SA (F1,20 = 9.34, p = 0.006), but no main effect of sex (F1,20 = 0.26, p = 0.618). Likewise,
there was a significant main effect of drug over the 5 IntAcc SA sessions (F1,20 = 21.21, p < 0.001), as well as a significant drug*sex interaction (F1,20 = 9.84, p = 0.005) whereby males obtained more infusions of fentanyl.

There was also a main effect of drug on number of active lever presses made over the last 5 sessions of ContAcc SA (F1,20 = 6.11, p = 0.023), and no main effect of sex (F1,20 = 0.10, p = 0.756). There were however significant session*sex, session*drug, and session*sex*drug interactions (F4,80 = 5.84, p < 0.001, F4,80 = 3.20, p = 0.017, and F4,80 = 3.33, p = 0.014 respectively), indicating that fentanyl self-administering male rats were continuing to escalate in number of active presses over time. Similarly, there was a significant main effect of drug on active presses (F1,20 = 14.21, p = 0.001), but no effect of sex (F1,20 = 0.71, p = 0.409) during the 3 drug-seeking tests, indicating that rats continually sought fentanyl throughout the two-week abstinence period.

As expected, there was no significant effect of drug or sex on number of inactive lever presses made during any phase of the experiment (all F’s < 3.94, all p’s > 0.061).
Figure 25. Infusions for drug across experimental phases (Exp 1).

Including responses made during cue-induced reinstatement of drug-seeking behaviour. Session 1-10 represents number of infusions obtained during ContAcc SA; session 11-15 represents number of infusions obtained during IntAcc SA; session 16-18 represents number of presses made on the drug-paired lever during cue-induced reinstatement. Fentanyl self-administering rats had increased responding for drug during all phases of the experiment *p < 0.05 compared to SAL. Data are presented as mean ± between-subjects SEM.

Experiment 2

5.3.1 Cued rGT Performance at Baseline

Rats displayed a clear preference for either optimal or risky choices on the cued rGT at baseline, as revealed by a significant main effect of risk preference on score (F1,39 = 14.90, p < 0.001), thus justifying our inclusion of risk preference as a between-subjects factor in subsequent analyses. Score distribution is shown in Figure 26. Likewise, there was a significant main effect of risk preference on trials completed (F1,39 = 5.25, p = 0.027), due to increased time out.
punishments acquired as penalty by risky rats. There was however no effect of sex on score or trials completed (F1.39 = 0.01, p = 0.917 and F1.39 = 2.44, p = 0.126, respectively).

There was no significant effects of sex or risk preference on choice latency, collection latency, omissions, or premature responding (all F’s < 2.84, all p’s > 0.100).

![Score distribution](image)

**Figure 26. Score distribution.**
Score distribution on the cued rGT at baseline (n = 48).

### 5.3.2 Fentanyl Self-Administration

As expected, rats allowed to self-administer fentanyl obtained more infusions and made more active presses than their saline self-administering counterparts. There was a significant overall main effect of both drug (Figure 27A; F1.35 = 23.36, p < 0.001) and sex (F1.35 = 13.77, p < 0.001) on number of infusions obtained over the last 5 sessions of self-administration. When looking only at fentanyl self-administering animals, there was both a significant main effect of sex (Figure 27B; F1.22 = 10.76, p = 0.003) as well as a risk preference*sex interaction (Figure
27C&D; F1,22 = 5.28, p = 0.031), whereby females, particularly those who were optimal
performers on the cued rGT, obtained more infusions of fentanyl than males. There was no
significant main effect of risk preference on infusions of fentanyl obtained (F1,22 = 0.72, p =
0.405).

Similarly, there was a significant main effect of both sex (F1,35 = 13.77, p < 0.001) and
drug (F1,35 = 12.15, p = 0.001) on number of active lever presses made over the last 5 sessions
of self-administration. When looking only at fentanyl self-administering animals, there was a
significant main effect of both risk preference (F1,22 = 4.50, p = 0.045) and sex (F1,22 = 12.58,
p = 0.002), as well as a significant risk preference*sex interaction (F1,22 = 8.90, p = 0.007),
whereby fentanyl self-administering females who were optimal performers on the cued rGT
made more active lever presses.

As expected, there was no significant effect of drug, sex, or risk preference on number of
inactive lever presses made during any phase of the experiment (all F’s < 2.32, all p’s > 0.137).
Figure 27. Infusions of fentanyl obtained (Exp 2).

A Fentanyl self-administering rats obtained more infusions of drug than saline self-administering rats. B Similarly, fentanyl self-administering females obtained more fentanyl infusions than males over the last 5 SA sessions. C While risk preference did not impact infusions of fentanyl obtained by males over the last 5 SA sessions, D we found that optimal females obtained more infusions of fentanyl than risky females over the last 5 SA sessions. *p < 0.05. Data are presented as mean ± between-subjects SEM

5.3.3 Concurrent Cued rGT and Fentanyl Self-Administration

There was no significant effect of drug on trials completed, score, choice latency, collection latency, omissions, or premature responding (all F’s < 0.33, all p’s > 0.571).

5.3.4 Effects of Fentanyl Withdrawal on Cued rGT Performance

When looking only at fentanyl self-administering animals, score decreased over the course of withdrawal (F12, 240 = 3.29, p < 0.001). There was a significant session*RP interaction (Figure 28A; F12,240 = 3.84, p < 0.001), indicating that optimal rats became riskier
during fentanyl withdrawal. Furthermore, there was a significant session*sex*RP interaction (Figure 28B&C; F12,240 = 2.00, p = 0.025), indicating that the observed decrease in score throughout fentanyl withdrawal was driven by males.

There was no significant effect of drug on trials completed, choice latency, collection latency, omissions, or premature responding (all F’s < 0.19, all p’s > 0.665).
Figure 28. Assessment of decision-making score across experimental phases in Exp 2.

Session 1-5 represents pre-SA baseline cued rGT performance; session 6-15 represents the 10 concurrent cued rGT and drug SA sessions; session 16-30 represents cued rGT performance throughout forced withdrawal. A Analysis of decision-making score of optimal animals by drug across experimental phases reveals that fentanyl self-administering rats get riskier throughout the course of fentanyl withdrawal. B Decision-making score for fentanyl-
taking males by risk preference reveals that this effect is driven by optimal males. C as decision-making score for fentanyl-taking females was not impacted by risk preference. *p < 0.05. Data are presented as mean ± between-subjects SEM

### 5.3.5 Reinstatement of Drug-Seeking

Animals previously allowed to self-administer fentanyl demonstrated pronounced cue-induced reinstatement of drug-seeking after a 21 day withdrawal period (Figure 29; F1,35 = 20.96, p < 0.001). There was no significant effect of sex or risk preference on fentanyl-seeking (all F’s < 1.68, p’s > 0.203).

![Figure 29. Reinstatement of drug-seeking behaviour by drug in Exp 2.](image)

Number of presses made on the drug-paired lever during cue-induced reinstatement of drug-seeking was increased in rats previously allowed to self-administer fentanyl. *p < 0.05. There was no significant main effect of sex or risk preference on reinstatement of drug-seeking. Data are presented as mean ± between-subjects SEM
5.4 Discussion

Here, we demonstrate a working model of fentanyl self-administration in both male and female Long Evans rats. Females trained on the cued rGT took more infusions of fentanyl than males, an effect that was particularly pronounced in optimal decision-makers. Contrary to previous reports using a similar experimental design with cocaine self-administration, neither male nor female rats increased preference for the risky options following fentanyl self-administration, regardless of whether they preferred the risky or optimal options at baseline. However, risky decision making significantly increased during forced withdrawal from fentanyl, an effect that was most pronounced in males.

Human addiction research indicates that females cycle through the stages of addiction more rapidly than males, as well as stabilize at higher levels of drug use (Anker & Carroll, 2010; Bobzean et al., 2014; Haas & Peters, 2000; Maria et al., 2014). Likewise, pre-clinical studies indicate that female rats self-administer opioids in greater amounts than their male counterparts (Carroll et al., 2001). This is congruent with our observation that females self-administer more fentanyl than males in Experiment 2. However, we first piloted the fentanyl self-administration protocol in behaviourally naïve rats (Experiment 1) and did not observe this sex effect.

The reasons why female rats may self-administer fentanyl more readily under some circumstances is currently unclear (see Little & Kosten, 2023 for review). However, the presence of sex differences in Experiment 2 but not Experiment 1 may be attributed to the impact of food restriction and cued rGT training on response to drugs of abuse. Firstly, it has been well established that food restriction increases the self-administration of drugs of abuse in both male and female rats (Carroll et al., 2001). Some studies have shown that while both males and females increase drug consumption under food restriction, this effect is mediated by the stress
response only in females (Carroll et al., 2001). Further studies indicate that the observed increase in drug-taking can be attributed to food restriction increasing the rewarding properties of abused drugs (Cabeza de Vaca & Carr, 1998; Zheng et al., 2012). Building off these observations, a multitude of studies have independently found females to have an increased sensitivity to the rewarding effects of drugs (Cullity et al., 2021; Karami & Zarrindast, 2008; Russo et al., 2003; Satta et al., 2018; Zakharova et al., 2009). These two findings may thus compound to produce the increased drug-taking seen in food-restricted females in our study.

Furthermore, repeated exposure to uncertain outcomes through probabilistic reinforcement schedules, using either Pavlovian or instrumental conditioning, can sensitize the dopamine system of male rats (Mascia et al., 2019; Singer et al., 2014; Zack et al., 2020; Zeeb et al., 2017). There is also evidence, albeit from a study using male rats only, that training on the cued rGT results in a relatively hypodopaminergic state at baseline, which may increase the reinforcing effects of the dopamine efflux produced by drugs of abuse (Ferland et al., 2019). An acute injection of cocaine also resulted in a greater elevation in dopamine release in these animals (Ferland et al., 2019). Studies investigating the role of sex in dopamine sensitization indicate that females show greater dopamine release in response to methylphenidate than males (Manza et al., 2022). It could thus be the case that experience in our rat gambling task potentiates the rewarding properties of fentanyl to a greater extent in females, thus explaining why we only found an effect of sex on fentanyl self-administration in Experiment 2.

Perhaps critically, the significantly greater fentanyl self-administration in female rats was driven by those making more optimal decisions in the cued rGT. The sex-specific nature of this finding may again be attributed to differences in dopamine signaling between males and females, in that chemogenetic manipulation of ventral tegmental area (VTA) dopamine neurons has
diametrically opposing effects on the evolution of decision-making patterns in the cued rGT across sex (Hynes et al., 2021, 2023). Specifically, chemogenetic inhibition of these cells increased risky choice in females while decreasing it in males (Hynes et al., 2021), whereas sensitizing these neurons through repeated chemogenetic activation increased risky choice in males while decreasing it in females (Hynes et al., 2023). Female optimal decision makers may therefore have higher basal levels of mesolimbic dopamine activity than males, which could theoretically increase the reinforcing effects of fentanyl. However, following this logic, we would also expect male risk-preferring rats to self-administer relatively more fentanyl, which we did not observe. While sex differences in dopaminergic signaling may play a role in determining the differential rates of fentanyl self-administration observed here, further research will be required to definitively test this hypothesis.

Whereas concurrent cocaine self-administration increases risky choice on the cued rGT, an effect that appears to be dependent on dopaminergic signaling in both sexes (Hynes et al., 2021, 2023), we did not note any acute decision-making changes as a result of fentanyl self-administration in our study. This is contradictory to previous literature, which suggests that higher levels of cognitive impairment are evident in individuals who use opioids, particularly in those who use heroin and/or fentanyl (Tamargo et al., 2021). However, cognitive deficits may have preceded drug use in these individuals. Indeed, other studies have found decision-making deficits in those with active stimulant but not opioid use disorders (Rogers, 1999). Nevertheless, we did note an increase in risky decision-making in optimal rats during fentanyl withdrawal. This is congruent with other findings, which report that decision-making deficits in male rats to persist in up to 7 weeks of abstinence from fentanyl (Wheeler et al., 2023). Research on newly-abstinent heroin users has similarly found impaired decision-making on the IGT, which was
alleviated by opioid maintenance treatment (Kriegler et al., 2019). In support of the sexually
dimorphic nature of our findings, other researchers have similarly found males to be more
sensitive to the effects of fentanyl withdrawal (Townsend et al., 2021). This could indicate that
females have greater levels of cognitive flexibility when it comes to coping with the cognitive
effects of opioid withdrawal, which may help them reallocate cognitive resources to other
behaviours during forced abstinence. Determining the neurobiological basis for withdrawal-
induced increases in risky choice in males may provide some insight into why males but not
females are more greatly affected.

It is important to note that 10 continuous short access sessions of fentanyl self-
administration is not capable of fully encapsulating the severity or complexity of human
addiction. Studies have demonstrated that while availability of drug on an IntAcc schedule
results in less cocaine-taking than when drug is available on a ContAcc schedule, it is more
successful at evoking addiction-relevant behaviours (Allain et al., 2017; Allain & Samaha, 2019;
Zimmer et al., 2012). Thus, Experiment 1 employed the use of IntAcc schedules in order to
assess whether these findings could be generalized to fentanyl-taking. Critically, the majority of
IntAcc methods use long-access (i.e. 6 hr sessions), rather than the short-access (i.e. 2 hr
sessions) employed here. However, there is evidence that short, IntAcc sessions are equally
capable of increasing addiction potential (Zimmer et al., 2012). The increased significance of
drug (i.e. saline vs fentanyl) in the IntAcc vs ContAcc phases of Experiment 1 do indeed support
the idea that IntAcc schedules increase addiction vulnerability, even when drug-taking sessions
are only 2 hours long. It will be important to determine whether IntAcc schedules result in
similar withdrawal-induced increases in risky choice in future experiments, and whether the
schedule of self-administration alters the cognitive sequelae of drug intake across sex.
In summary, this research highlights the importance of looking at both males and females when assessing any addiction-relevant behaviours, as well as the fact that not all drugs of abuse have uniform effects on cognition. Furthermore, our finding that decision-making deficits are apparent during fentanyl withdrawal may explain why relapse is so common amongst opioid users, and could thus be used to inform treatment in newly-abstinent individuals.
Chapter 6: General Discussion

6.1 Summary of Experiments

The experiments outlined in this dissertation take multiple different approaches to addressing the link between cue-induced risky decision-making and addiction-relevant behaviours. First, I demonstrated the influence of histone acetylation on decision-making in the cued rGT. In Chapter 3, I found that systemic administration of the HDAC inhibitor NaBut increased risky decision-making specifically during acquisition of the cued rGT. I also showed that NaBut altered how rats learn from task outcomes. Specifically, animals treated with NaBut simultaneously showed enhanced learning from wins and reduced learning from losses. This is similar to learning deficits induced by the presence of cues when comparing rats trained on the cued vs uncued rGT (Langdon et al., 2019). Our results also point to the role of habit formation in risky decision-making, as observed risk-taking was accompanied by increased histone acetylation in brain regions that mediate habit.

I also found that salient, reward-paired audiovisual cues have a similarly detrimental effect on rGT performance. Our lab has previously shown that rats trained on the cued rGT are riskier and more impulsive than rats trained on the uncued rGT (Barrus & Winstanley, 2016; Ferland et al., 2019; Hathaway et al., 2022). This is corroborated by Chapter 4 findings, which further indicate that this effect is observed in a within-subjects design whereby rats can choose between the cued and uncued rGT on a trial-by-trial basis. This indicates that cues cause decision-making and impulse control deficits in a transient nature, as opposed to being a lasting result of being trained on the cued rGT. I also found that while risk-preferring rats had a greater preference for the cued task at baseline, this preference was abolished after the acquisition of
cocaine-taking. This demonstrates a potential trade-off between two forms of dopamine-evoking tasks: cued gambling and cocaine-taking.

I further show that cognitive effects of addiction-relevant behaviours are not shared amongst all drugs of abuse. In Chapter 5, I found that while females self-administer more fentanyl, neither sex is impacted by the acute effects of fentanyl self-administration. We had originally hypothesized that fentanyl self-administration would mirror decision-making deficits previously seen by our group in response to cocaine self-administration (Ferland et al., 2019; Ferland & Winstanley, 2017). While we did not confirm this hypothesis, our findings are supported by other studies which find decision-making deficits in those with active stimulant but not opioid use disorders (Rogers, 1999). I did however demonstrate that fentanyl withdrawal does induce decision-making deficits on the cued rGT. This is in line with previous research which demonstrates more severe cognitive impairments resulting from opioid withdrawal rather than active opioid use (Dalley et al., 2005; Jamison et al., 2003; Rapeli et al., 2006; Wheeler et al., 2023).

6.2 Theoretical Implications

It is known that the presence of cues increases risk-taking on the rGT, however the neurocognitive mechanisms underlying this effect were previously unclear. In Chapter 3, I show that the development of a maladaptive, risky decision-making strategy during acquisition of the cued rGT can be facilitated through neurobiological mechanisms that enhance habitual control of behaviour – namely, HDAC inhibition. In Chapter 4, I show that cue-induced deficits are transient in nature, as they specifically impact decision-making on cued trials and not subsequent uncued trials. Furthermore, results from Chapter 4 demonstrate that the presence of cues is
preferred to their absence. This dispels the alternative hypothesis that observed cue-induced
decision-making deficits are a result of increased stress imposed by cues.

Our findings from Chapter 3 may also have implications in the pathology of addiction,
wherein cues gain salience and promote further drug-taking or engagement in gambling.
Previous research provides support for both dysregulated habit formation and goal-directed
behaviour in the etiology of addiction. Our findings from Chapter 3 show support for the former
hypothesis, that is that cues interact with the formation of habits, and demonstrate that this can be
mediated by histone acetylation in striatal subregions.

While I did not directly manipulate or measure neurotransmitter levels in the experiments
included in this dissertation, our current findings may be in line with previous results that cue-
evoked dopamine release induces a hypodopaminergic state over time, while simultaneously
increasing dopamine efflux in response to reward. This hypodopaminergic state may then drive
increased risk-taking, in accordance with the reward deficiency theory of addiction. In support,
many of the effects observed in this dissertation, such as increased risk-taking and drug use, are
known to implicate dopaminergic signaling. Indeed, exposure to reward-paired cues, drugs of
abuse, and gambling have all been shown to cause lasting alterations in dopamine (Ferland et al.,
2019; Kayser, 2019; Willuhn et al., 2014), which may thus act synergistically to confer the
addiction vulnerability demonstrated in this dissertation.

6.3 Limitations and Future Directions

One major limitation in this dissertation pertains to the investigation of sex differences.
Our tasks require large cohort sizes due to the presence of large individual differences in baseline
decision-making profiles. Because of this, we did not include females in Chapter 3. Running this
paradigm again in females would yield important information about how histone acetylation may
differentially affect rGT performance in females. Indeed, there is some evidence to suggest that estrogen may interact with HDAC activity in females to increase reward sensitivity (Torres, 2022), which may translate to altered response to reward-paired cues. Furthermore, while behavioural sex differences are investigated and found to exist in Chapters 4 and 5 of this dissertation, mechanistic sex differences remain to be investigated. Additionally, while some behaviours are found to be consistent between sexes, there may be different underlying neurocognitive processes that cause this common behaviour, which was not able to be assessed with the methods used here. Indeed, there is evidence suggesting this to be true in both cognitive bias and reward processing (Orsini, Brown, et al., 2022). Therefore, future studies are required to further elucidate the role of sex in the bi-directional relationship between decision-making and addiction vulnerability.

Another limitation of this dissertation pertains to the self-administration paradigms utilized here. We primarily used 10 sessions of short access (2 hr) to drug available on a fixed ratio-1 schedule of reinforcer availability to model of addiction-relevant behaviour. While this paradigm has been historically considered to be the gold-standard, it fails to encapsulate multiple domains of impairment seen in human addiction, such as drug use in the face of negative consequences and the prioritizing drug use over other life domains or activities. In line with this, there has been increasingly research aimed at better modeling the multi-faceted impairments seen in human addiction. For example, researchers have found that when given the choice between social and drug reward, the majority of rats prefer the social reward (Venniro et al., 2018, 2021). This mirrors how only a select number of individuals who engage in drug-taking go on to develop a substance use disorder. Thus, a more appropriate model of addiction may be to allow animals the choice between a drug and non-drug reward. Based on our findings that the majority
of rats prefer to engage with the cued vs uncued rGT, we have now developed another novel behavioural task that allows animals to choose between the cued rGT and self-administration of cocaine on a trial-by-trial basis. We hope to use this paradigm in future studies to better model the multi-faceted nature of human addiction, and to understand the unique differences that exist in rats who continue to choose drug-taking over other salient and rewarding tasks.

Future experiments are also required to understand the mechanism of fentanyl withdrawal-induced decision-making deficits. I, in collaboration with past lab members, previously employed chemogenetics to understand the contribution of VTA dopaminergic signaling to both performance on the cued rGT and cocaine self-administration (Hynes et al., 2020, 2021). Future studies could thus employ similar techniques in order to assess the contribution of dopamine signaling to fentanyl self-administration and withdrawal-induced decision-making deficits. Furthermore, recent clinical studies have found that increased tolerance of ambiguity is the most salient predictor of imminent relapse in past opioid users (Konova et al., 2020). Future studies could therefore seek to determine neural underpinnings of this phenomenon in animal models.

In order to better understand why some individuals are more vulnerable to addiction than others, we have also established a rat model of the two-hit hypothesis. The two-hit hypothesis posits that an early life immune challenge leaves individuals uniquely vulnerable to developing neuropsychiatric disorders in adulthood when followed by a second “hit” in adolescence. As our first “hit,” we administer the endotoxin lipopolysaccharide at post-natal days 4 and 6. We then investigated multiple forms of second “hits,” including another systemic immune challenge of lipopolysaccharide, oral cannabis oil, or acute restraint stress at post-natal day 30. We then euthanized these animals at varying timepoints in order to assess levels of pro-inflammatory
gene expression changes. We thus plan to extend this model by evaluating the performance of these animals in the cued rat gambling task and cocaine/fentanyl self-administration paradigms to determine how decision-making and addiction vulnerability is altered under the two-hit hypothesis of early life immune challenge. We are also interested in assessing how traumatic brain injury, a potent inducer of neuroinflammation, may serve as a second “hit” in this model.

6.4 Concluding Remarks

The experiments presented in this dissertation provide further evidence that the presence of salient, reward-paired audiovisual cues are detrimental to decision-making, the role of HDAC activity in this relationship, and how decision-making is differentially regulated by different drugs of abuse and biological sex. The novel behavioural paradigms we have developed can be used in future experiments to further elucidate the effects of cues and drug manipulations on decision-making. Furthermore, the results presented here can be used to better inform the treatment of addiction, particularly as cocaine-induced motivational deficits and decision-making deficits that arise during fentanyl withdrawal are likely to contribute to treatment drop out and high rates of relapse.

Those with addiction carry a heavy burden. In extreme cases, the combination of drug use and cognitive differences contribute to these individuals losing everything they have, from their homes and belongings to their loved ones and support network. The stigma associated with these conditions, combined with limited availability of resources and overall lack of effective treatment options, poses a huge challenge to recovery. By gaining a better understanding of the neurocognitive mechanisms of decision-making deficits and addiction, I hope to inform how future treatments can better support recovery and the maintenance of abstinence, and thus allow these individuals to regain control over their future.
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