FACTORS INFLUENCING REWARD-SEEKING BEHAVIOUR IN RATS AND THE IMPLICATIONS FOR PROBLEM GAMBLERS

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DEDICATION

This is dedicated to my family and friends, for the endless support and love, and especially to my dog, Amy, for being such a good girl.

ABSTRACT

Depression and impulsivity have been repeatedly implicated in gambling pathology, but the relationship between these factors is not fully understood. There is evidence of overlapping neural circuitry that may explain the relatedness of these disorders. The following thesis will characterize the neural dysfunction of gambling addiction, depression, and impulsivity, and will argue for the use of animal models to further our understanding of these relationships. Two series of experiments were conducted to examine how these factors influence reward-seeking behaviour. In the first, we will see that depression can lead to compulsive reward-seeking in rats; and in the second, we will present evidence that proves just how motivating gambling-like schedules of reinforcement truly are, and what that means for impulsive problem gamblers.

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CHAPTER 1: INTRODUCTION

Gambling disorder is a behavioural addiction that is estimated to effect 0.6% of the Canadian population, while 2.7% of Canadians are at risk of developing gambling problems in the future (Williams et al., 2020). It is characterized by having an intense preoccupation with gambling, seeking gambling activities when upset or stressed, needing to bet in excess to feel the same excitement, chasing losses after losing money, repeated unsuccessful attempts to gain control over one's gambling which tends to be accompanied by feelings of irritability or restlessness when trying to cut down, needing to rely on others because of money problems associated with gambling, lying about gambling activity, and losing close relationships or employment opportunities because of gambling (American Psychiatric Association, 2013). Treating gambling addiction requires the consideration of concurrent mental health diagnoses: it is estimated that as many as 96% of problem gamblers have at least one comorbid DSM diagnosis (Kessler et al., 2008), the most common being lifetime substance use disorders and lifetime mood disorders (Crockford & el-Guebaly, 1998; Hodgins, Peden, & Cassidy, 2005).

Making matters more complex, research suggests that the causes of gambling disorder may be different for different people, and therefore several subtypes of problem gamblers have been proposed. Among the first categorization schemes was that of Moran (1970), where he separated 50 male problem gamblers into five categories: impulsive, characterized by excessive gambling and loss of control; neurotic, who gambled to relieve feelings of stress; psychopathic, where gambling was a result of antisocial behaviour; symptomatic, who suffered from problem gambling as a result of another condition (typically a mood disorder); and subcultural, where they experienced pressure from the people around them. From here, new categories were proposed: recurringly depressed and chronically under-stimulated (McCormick, 1988); escape seekers and

action seekers (Lesieur & Blume, 1991); psychological distress, sensation seeking, impulsive antisocial, and crime and liveliness (Steel & Blaszczynski, 1996). One of the most popular, the pathways model of problem gambling, proposed three subtypes: behaviourally conditioned, emotionally vulnerable, and antisocial impulsivist (Blaszczynski & Nower, 2002). The behaviourally conditioned subtype is comprised of gamblers lacking premorbid psychopathology. People in the emotionally vulnerable subtype have comorbid depression and/or anxiety, poor coping skills, and often seek out gambling as a form of an escape. The final pathway, the antisocial impulsivist subtype, includes those with elevated rates of impulsive, attention deficit and antisocial behaviour. Milosevic and Ledgerwood (2010) summarized all of these attempts at subtyping over the years, and found that most of the subtypes proposed over time could be ascribed to one of the subtypes defined in the pathways model of problem gambling.

A recent systematic review found that most studies that used empirical measures and statistical tests to subtype pathological gamblers found three clusters, similar to the ones proposed by Blaszczynski and Nower (Kurilla, 2021). However, not all studies that found three clusters were able to completely validate all three pathways (Valleur et al., 2016), some proposed less pathways (Lobo et al., 2014), some proposed more (Black & Allen, 2021; Huggett et al., 2021), and the pathway that seemed the least consistent was the emotionally vulnerable pathway (Kurilla, 2021). Regardless, there have been plenty of studies that have validated all three categories of the pathways model of problem gambling (Mader, Christensen, & Williams, 2019; Moon, Lister, Milosevic, & Ledgerwood, 2017; Nower & Blaszczynski, 2017; Nower, Martins, Lin, & Blanco, 2013), with depression and impulsivity always being strongly implicated as factors influencing the development of gambling addiction.

It should be noted that while the pathways model is the most popular model, it may not be the most ecologically valid model; many elements contribute to the development of problem gambling and the pathways model may be too simplistic. Longitudinal studies have identified other factors that can increase the likelihood of experiencing disordered gambling: the intensity and type of gambling; personality factors like higher neuroticism, lower agreeableness and lower conscientiousness; lower IQ and educational attainment; family history of mental health diagnoses; or experiencing a large win while gambling (Williams et al., 2015; Williams, Volberg, Zorn, Stanek, & Evans, 2021). A better model would incorporate these factors and approach gambling addiction from a more biopsychosocial perspective.

Despite the simplicity of the pathways model, the factors it implicates do appear to play an important role in the formation of problem gambling. The following thesis will look at characteristics of the emotionally vulnerable and antisocial impulsivist pathways, depression and impulsivity respectively, how these can influence reward-seeking behaviour in rats, and what that might mean for humans.

The Emotionally Vulnerable Pathway & Depression

Like gambling addiction, depression is complex and the neural mechanisms underlying it are not fully elucidated. There are many theories about the underlying cause of depression (changes in monoamine transmission, the HPA axis, inflammation, or neuroplasticity) and emerging evidence suggests that these theories likely overlap (Dean & Keshavan, 2017).

Altered levels of serotonin, norepinephrine and dopamine have all been hypothesized to play a role in depression. The discovery that monoamine oxidase inhibitors and tricyclic agents, which inhibited serotonin, norepinephrine and dopamine reuptake transporters, could treat

depression paved the way for the monoamine theory of depression, and for the development of antidepressants like serotonin-selective reuptake inhibitors (Nestler et al., 2002). Monoamines were implicated in depression because of this, and while these medications are still readily prescribed, these antidepressants are not effective for everyone and their success rate is only ~50% (Nestler et al., 2002). Dopamine has been lesser implicated but has still received a bit of attention for its role in anhedonia and reduced motivation (Dean & Keshavan, 2017), and for reports of improving mood in people taking dopamine agonists (Leentjens, 2011). It is no surprise that all these monoamines have been linked to some degree of improvement because these systems modulate each other: dopaminergic neurons in the ventral tegmental area can modulate noradrenergic neurons in the locus coeruleus and vice versa, while both areas can also modulate serotonergic neurons in the dorsal raphe nucleus (El Mansari et al., 2010).

Stress can cause dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, which has been strongly implicated in the pathology of depression. Childhood stress, like neglect or abuse, drastically increases the likelihood of developing depression later in life (Dean & Keshavan, 2017; Juruena, 2014). Putting animals through a chronic mild stress paradigm, where they are continually exposed to mild stressors (like wet bedding, inconsistent light cycles, and noisy conditions), induces depression-like behaviours; these same results can be achieved through chronic corticosterone administration (Willner, 2017) and can lead to reduced brain plasticity (Dean & Keshavan, 2017). People with depression have been repeatedly shown to have higher levels of salivary cortisol (Cowen, 2010). While this supports the idea that stress leads to depression, it is also possible that depressed people simply experience more stress. Further supporting the role of stress in causing depression, one stressful life event can increase the risk of a major depressive episode 1.41 times, and chronic stress has been linked to worse prognoses, treatment resistance

and higher frequency of relapse (Liu, Liu, Wang, Zhang, & Li, 2017). When stress disrupts the HPA axis, the HPA axis can lose its ability to modulate neuroinflammation (Jeon & Kim, 2018).

Inflammation is also thought to contribute to depression. People with depression often have more inflammatory markers and there is mounting evidence that cytokines disrupt the monoamine systems, stimulate the HPA axis, and can have adverse effects on neuroplasticity and neurogenesis, especially if the presence of neurotrophic factors like BDNF, which promotes survival and growth of neurons, is already low (Felger & Lotrich, 2013; Jeon & Kim, 2018; Song & Wang, 2011). Artificially increasing inflammatory markers, via methods like stress or by inducing an immune response (i.e., through typhoid fever vaccination or hepatitis C treatments; Wichers et al., 2007; Wright, Strike, Brydon, & Steptoe, 2005), can temporarily induce a depressed mood, and autoimmune or inflammatory disorders have been correlated with depression (Dean & Keshavan, 2017; Jeon & Kim, 2018). One meta-analysis found that anti-inflammatory drugs (like NSAIDs or omega-3 fatty acids) significantly improved symptoms of major depressive disorder (Bai et al., 2020). There is also evidence that selective-serotonin and -norepinephrine reuptake inhibitors may have anti-inflammatory properties (Dionisie, Filip, Manea, Manea, & Riga, 2021).

Another key element of the neurobiology of depression is decreases in neurogenesis, especially in the hippocampus, which can also be caused by chronic stress in humans and animals (Cowen, 2010; Kornhuber & Gulbins, 2021). Brain-derived neurotrophic factor, or BDNF, is lower in people with depression, which is interesting as BDNF plays a vital role in neuroplasticity, and because antidepressants may increase BDNF: ketamine can increase BDNF in hippocampal pyramidal cells, while fluoxetine increases BDNF in the dentate gyrus (Dean & Keshavan, 2017). There is also evidence that other antidepressants can increase neurogenesis in the hippocampus (Santarelli et al., 2003). Post mortem analysis of people with Parkinson's disease, and animal

models of Parkinson's disease, have shown degeneration in not only the substantia nigra, but also reduced volume in the hippocampus, which some researchers have suggested may contribute to the development of comorbid depression (Lim, Bang, & Choi, 2018).

Studies have shown that depression can act as a predictor of gambling severity (Hounslow, Smith, Battersby, & Morefield, 2011) and as a mediator in the susceptibility to cognitive distortions (Levesque, Sevigny, Giroux, & Jacques, 2018). Some people are drawn to gambling activities as a way to cope with their negative emotional state (MacLaren, Ellery, & Knoll, 2015). The reinforcing stimuli from slot machines, coupled with pre-existing sensitivity to boredom and the proneness to seek escape (Dixon et al., 2019), leads to a "dark flow" state, where one feels completely immersed in the gambling experience (Dixon et al., 2018).

The Antisocial Impulsivist Pathway & Impulsivity

Impulsivity is often broken down into two constructs: choice impulsivity (preference for immediate rewards) and motor impulsivity (response inhibition). Delay discounting tasks are one of the most popular ways to measure choice impulsivity. Participants (human or otherwise) are given the option between a small, immediate reward or a large, delayed reward. The delay is increased, and the measure is how quickly they switch from the large reward to the small reward (Ainslie, 1975; Mar & Robbins, 2007). The 5-choice serial reaction time task, or 5CSRTT, is a popular measure of motor impulsivity. In this task, participants must withhold a response while maintaining attention, and then select the cued option after a brief delay. Researchers have suggested that the proportion of premature responding, or responding before the cue, is indicative of motor impulsivity (Dalley & Robbins, 2017).

Monoamine systems are thought to exert regulatory power over impulsivity: dopamine's role is strongly implicated by the use of amphetamines and methylphenidate to treat attention-deficit/hyperactivity disorder; atomoxetine administration, a selective-norepinephrine reuptake inhibitor, has been successfully used to treat impulsive behaviour in many rodent paradigms (Dalley & Robbins, 2017); adrenergic receptor agonists, such as guanfacine, increase macaques' choice of the delayed reward (Kim, Bobeica, Gamo, Arnsten, & Lee, 2012); and selective-serotonin reuptake inhibitors (SSRIs) have also been reported to reduce the severity of various impulse control disorders and problem gambling in some patients (Hollander & Rosen, 2000).

The prefrontal cortex, orbitofrontal cortex and striatum are all implicated in impulsivity (Dalley, Mar, Economidou, & Robbins, 2008; Winstanley, Eagle, & Robbins, 2006). Choice impulsivity has been repeatedly associated with an overactive reward drive, seen via increased activity in the medial prefrontal cortex and the ventral striatum (Grant & Kim, 2014). Paradoxically, lesions in the ventral striatum (nucleus accumbens) in rats increase delay discounting (Winstanley et al., 2006), Within the nucleus accumbens, the core region seems to be key because lesions restricted to this subregion also increase impulsivity in rats (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). Further supporting the role of the nucleus accumbens in impulsivity, lesions to the basolateral amygdala, which is strongly connected to the nucleus accumbens, also increase delay discounting (Winstanley, Theobald, Cardinal, & Robbins, 2004). The orbitofrontal cortex has also been implicated in impulsivity. In rats, lesioning the orbitofrontal cortex and subthalamic nucleus decreases delay discounting (Winstanley et al., 2006). On the other hand, people with orbitofrontal cortex damage perform worse in a decision-making assessment known as the Iowa gambling task. In this task, they tend to favour the large reward/large punishment option, which is less advantageous, and also thought of as the more

impulsive option (Bechara, Damasio, Damasio, & Anderson, 1994). Winstanley (2006) suggests that the effects of orbitofrontal damage in rodents and humans are complimentary, as both samples preferred the larger magnitude reward in their respective tasks.

Meta-analyses looking at various addictions and delay discounting have found that people who meet the criteria for addiction have greater rates of delay discounting (MacKillop et al., 2011) and that addiction severity is positively correlated with the steepness of one's delay discounting curve, or their k value, although this effect size may be small (Amlung, Vedelago, Acker, Balodis, & MacKillop, 2017). Another study found that age, gender, education, history of substance abuse treatment, and smoking history failed to significantly predict k values, but that the South Oaks Gambling Screen had 1.4 times higher predictive value for delay discounting behaviour than the Eysenck Impulsivity Scale (Alessi & Petry, 2003), suggesting that gambling propensity, as measured by the SOGS, is strongly related to choice impulsivity. Delay discounting was significantly correlated with susceptibility to cognitive distortions, as measured by the Gambling-Related Cognitions Scale, in both problem gamblers and healthy controls (Michalczuk, Bowden-Jones, Verdejo-Garcia, & Clark, 2011). All problem gamblers have higher rates of delay discounting, and because it is a characteristic strongly shared by the entire group, it might not indicate severity like other measures can (D. Brevers et al., 2012). Does this mean that problem gamblers become impulsive, or that impulsive people become problem gamblers? Longitudinal studies have suggested that those who are impulsive are more likely to subsequently suffer from gambling addiction (Vitaro, Arseneault, & Tremblay, 1997, 1999; Vitaro, Ferland, Jacques, & Ladouceur, 1998), but more work should be done as the criteria for sufficient participation in gambling activities was low.

Your Brain on Gambling

Gambling addiction was classified as an impulse control disorder until the most recent edition of the *Diagnostic and Statistical Manual of Mental Disorders*, the DSM-V, was published (American Psychiatric Association, 2013); its characterized by underactivity in the orbitofrontal cortex, ventromedial prefrontal cortex and ventral anterior cingulate cortex, and hyperactivity in the ventral striatum and nucleus accumbens (Fineberg et al., 2010; Potenza, 2008). The ventral striatum and nucleus accumbens shell are thought to be responsible for the primary reinforcing effects of drugs of abuse (Everitt et al., 2008). The HPA axis seems to be influenced in problem gambling as well, with plasma cortisol levels negatively predicting gambling severity (Geisel, Panneck, Hellweg, Wiedemann, & Muller, 2015).

Given the information above, it is unsurprising that the monoamine system in people with gambling addiction also appears to be dysfunctional. Norepinephrine is thought to play a role in excitement and arousal, dopamine is significant for rewards, learning and reinforcement, and serotonin for the initiation and cessation of behaviours (Potenza, 2008). Dopamine plays an interesting role, as exemplified by the fact that Parkinson's patients on dopamine agonist therapy show rates of gambling addiction that are dramatically increased (Ambermoon, Carter, Hall, Dissanayaka, & O'Sullivan, 2011; Weintraub et al., 2010) and that dopamine synthesis capacity, or the rate of dopamine turnover, is positively correlated with gambling severity (van Holst et al., 2018). Interestingly, as a drug's affinity for the D3 receptor (over the D2 receptor) increases, so do the instances of impulse control disorders and problem gambling associated with that drug (Seeman, 2015). Though not a monoamine, opioids also contribute to the pathology of gambling addiction, and are important for pleasure; naltrexone and nalmefene, opioid antagonists, have shown some success in the treatment of gambling disorder (Antons, Brand, & Potenza, 2020; Di Ciano & Le Foll, 2016).

There is evidence of four networks that influence the development and maintenance of addiction: the reward network, consisting of the amygdala, orbitofrontal cortex, ventral striatum, and rostral anterior cingulate cortex; the executive control network, which includes the dorsolateral, dorsomedial and ventrolateral prefrontal cortex; the salience network, including the inferior parietal lobe, dorsal anterior cingulate cortex, and the insula; and finally, the habit network, which consists of the caudate nucleus and the putamen (Antons et al., 2020). Different fronto-striatal representations are associated with the steeper discounting curves that are significantly found in problem gamblers: during delay discounting, problem gamblers show greater activity in the rostral anterior cingulate cortex, the right orbitofrontal cortex, and the ventral striatum (Punia & Balodis, 2019). One fMRI study showed that problem gamblers have stronger integration of the right middle insula, an area theorized to influence addictive drive, in the ventral attention network, and that cognitive distortions are associated with stronger medial prefrontal cortex, amygdala and insula integration in the resting-state networks (van Timmeren, Zhutovsky, van Holst, & Goudriaan, 2018).

Models for Studying Gambling Addiction in Animals

While progress is being made in understanding the pathology of gambling addiction, there is not enough known about the neural correlates underlying this disorder to develop pharmacological interventions or provide effective treatments for everyone who is suffering. Animal models provide an interesting opportunity to study the brain in ways that are not feasible with human participants. Although gambling addiction is a complex disorder, often touted as "too complex" to be studied in animals, much has already been learned about the neural mechanisms of gambling using animal subjects.

The aforementioned Iowa gambling task (IGT) is a very popular way to assess decision-making under risk in humans. In the IGT, participants must optimize reward collection by selecting between four decks: two that pay out less but have smaller losses, two that pay out more but include larger losses. The safer, small option pays out more over time, despite the smaller wins. While most people learn the task and choose the optimal strategy, there is a subset of the population that persists in choosing the riskier option. Gambling severity has been associated with poorer decision-making within this paradigm (Damien Brevers et al., 2012). The rodent gambling task (rGT) was developed as an animal version of this task (van den Bos, Lasthuis, Den Heijer, Van der Harst, & Spruijt, 2006) and is currently the most widely-used model of risky decision-making in rodents. In the rGT, rodents choose between levers that use time delays, quinine pellets or foot shock as punishment, rather than monetary losses. Like humans, most rats learn to make optimal choices, but there remains a subset of the population that do not (Rivalan, Ahmed, & Dellu-Hagedorn, 2009). One characteristic of the human emotionally vulnerable subgroup of problem gamblers is that they often have experienced adverse life events. Similarly, a study in rats found that while a pre-existing preference for risky decision-making, as measured by baseline rGT performance, was unable to predict the degree to which a rat developed learned helplessness, exposure to the learned helplessness protocol did result in subsequent deficits in rGT performance (Nobrega, Hedayatmofidi, & Lobo, 2016). This finding is particularly interesting when considering that studies have shown that major depressive disorder in adolescents is also associated with poorer Iowa gambling task performance (Han et al., 2012).

Probabilistic and delay discounting tasks provide further methods for disentangling features of problem gambling, specifically impulsivity and advantageous decision-making. In probabilistic delay tasks (rPDT), rats choose between a small, certain reward or a larger reward

with a decreasing probability of success (Cardinal & Howes, 2005). In delay discounting tasks (rDDT), the idea is the same except using increasing time delays instead of decreasing probabilities (Mar & Robbins, 2007). In both tasks, researchers are interested in how quickly animals change from the large reward to the small reward as a function of delay or probability. These tasks have strong translation potential, as they have been successfully used to evaluate both animals and humans. In the human literature, impulsivity has been strongly linked to the severity of gambling addiction (MacLaren, Fugelsang, Harrigan, & Dixon, 2012). Increased impulsivity, as measured in the rDDT, has been correlated with compulsive water drinking in schedule-induced polydipsia procedures (Ansquer et al., 2014), increased cocaine self-administration (Perry, Larson, German, Madden, & Carroll, 2005) and may be correlated with preference for gambling-like schedules of reinforcement (Belin, Mar, Dalley, Robbins, & Everitt, 2008; Madden, Ewan, & Lagorio, 2007).

The rodent slot machine task (rSMT) was created to study the effects of near-miss trials in animals (Winstanley, Cocker, & Rogers, 2011). A near-miss is any situation where the outcome is close to a win but is not a win: such as when the last icon on a pay-line does not match, leaving the player one icon away from a win. In the rSMT, rats are presented with two levers: one to spin and one to collect. Once the rat initiates a spin, lights will indicate whether the animal can receive reward or not. All four lights illuminated signifies a clear win and the rat can receive its reward by pressing the collect lever. One light illuminated is a clear loss, two is a near-loss and three is a near-miss; the animals cannot collect reward on any of these trials and must re-spin. In humans, near-misses have been shown to increase skin conductance, which is physiological evidence of the frustrating effects of being *so close* to winning; but paradoxically, this aggravating structural feature of slot machines increases motivation to continue playing, as indicated by reduced latency to initiate the next spin (Dixon, MacLaren, Jarick, Fugelsang, & Harrigan, 2013). Near-misses

have also been shown to activate areas of the brain, such as ventral striatum and anterior insula, in a similar manner to wins, which may accelerate the propensity to continue gambling (Clark, Lawrence, Astley-Jones, & Gray, 2009).

Losses-disguised-as-wins (LDWs) are another structural feature of slot machines that are thought to increase play. An LDW occurs when the amount won is less than the amount wagered. Players show increased arousal to LDWs, similar to that of win, despite the net loss that occurs on these trials (Dixon, Harrigan, Sandhu, Collins, & Fugelsang, 2010). The rodent LDW task (rLDWT) is used to examine this effect in animals (Ferland et al., 2018). In this task, rats choose between a certain option (yielding two pellets) or an uncertain option, with a 50% of being rewarded, except 35% of the wins result in three pellets instead of four (LDW). LDWs increase arousal and make losing streaks less noticeable (Dixon et al., 2010). In the rLDWT, most rats switch to the more advantageous option (the certain option) as LDW frequency is increased, although there is a subgroup of animals that appear vulnerable to LDWs and do not improve despite the diminishing rate of return (Ferland et al., 2018). In both humans and rats, the presence of LDWs seems to interfere with the ability to track losses over time, ultimately leading to disadvantageous decision-making.

The rodent betting task (rBT) is a way to measure wager sensitivity bias, or irrational choice under uncertainty. Rats must choose between two levers: one offering a sure reward and one offering “double or nothing”. Wager size is manipulated but the potential payout over multiple trials is equal across levers. As the wager size increases, a subgroup of wager-sensitive animals emerge, and these animals dramatically switch their preference to the safe lever, although it has no benefit (Cocker, Dinelle, Kornelson, Sossi, & Winstanley, 2012). Unlike methods like rGT, where the larger option is always less advantageous, in this task both options result in the same

amount of total reward, so a rational rat should not express a preference for one option over the other. Hence, animals who become biased to the safe option as uncertainty increases are behaving irrationally. Susceptibility to erroneous cognitions, especially under risk, is a predictor of vulnerability to problem gambling in humans (Michalczuk et al., 2011), leading Cocker et al. to suggest that the irrational, risk-averse rats are analogous to human problem gamblers; however, human studies have shown that problem gamblers, rather than seeking safety, tend to be more risk prone (Ligneul, Sescousse, Barbalat, Domenech, & Dreher, 2013). Further, healthy people show a shift towards safety as bet size increase, a trend that also looks irrational through the lens of normative economic theory (Ligneul et al., 2013; Tversky & Kahneman, 1992).

One more feature of problem gambling is the urge to chase losses, motivated by the hopes of breaking even. This phenomenon can be studied using the rodent loss chasing task (rLCT) (Rogers, Wong, McKinnon, & Winstanley, 2013). In the rLCT, rats press a lever to win or lose. If the rat loses, which occurs 30% of the time, it can choose to chase or quit. Quitting starts a brief time out (four seconds) and then the animal can initiate the next trial. Chasing results in a 50% chance of winning or a 50% chance of experiencing a longer time out (eight seconds). If the rat loses again, it can continue to chase – this time, at the risk of experiencing a 16 second time out – or quit. The time out period doubles each chase, until the rat wins or quits. Both rats (Cocker & Winstanley, 2015; Rogers et al., 2013) and humans (Lesieur, 1979) are biased towards chasing losses, and tend to be willing to risk "double or nothing", even when that bias leads to less reward overall.

Experimental Objectives

The studies highlighted here have demonstrated that impulsivity and depression may have shared neural substrates with gambling addiction, which could explain the high comorbidity of

these disorders. Unfortunately, we still do not understand the relationships between these factors and the pathology of gambling addiction. Many aspects of the gambling experience have been successfully disentangled using animal models, which are necessary both to continue to learn about these disorders and to test new interventions. The following chapters will look at how two factors, depression and impulsivity, can influence reward-seeking behaviour in rats, and what this means for people with gambling addiction. This thesis examined these factors in two separate sets of experiments.

The first set of experiments tested whether addiction-like behaviour induced by prolonged exposure to a gambling-like reward schedule would be more likely in rats that are considered a genetic model of depression (Wistar-Kyoto rats). The objective was to examine how depressive behaviour, as tested using this animal model of depression, influenced reward-seeking behaviour under two schedules of reinforcement: the random-ratio (gambling-like) schedule and the fixed-ratio schedule. Our hypothesis was that animals with a depressive genotype on a random-ratio schedule will show higher motivation to obtain reward, culminating in increased response rates, shorter latencies to initiate the next trial after being rewarded, and persistent responding despite increasing work requirements. These animals will also show higher levels of compulsive behaviour, which will manifest in the form of more responses when reward is cued as unavailable, persistent responding despite increasingly negative consequences, and a higher propensity to respond after a period of abstinence.

The objective of the second set of experiments was to examine the relationship between choice impulsivity and preference for gambling-like schedule of reinforcement. More specifically, we sought to determine if impulsivity could predict the degree to which a rat preferred being rewarded on a random-ratio schedule. We hypothesized that animals that are more impulsive, as

assessed by steeper discounting curves (or greater preference for the smaller, immediate reward) within a delay discounting paradigm, will show a greater attraction to random-ratio schedules of reinforcement over fixed-ratio schedules with the same average reward frequency.

CHAPTER 2: DEPRESSION

Introduction

People seek out gambling activities for many different reasons and the underlying psychopathology varies from one problem gambler to the next. Consequently, it is common for researchers to classify problem gamblers by subtype. One group that is consistently included within these explanatory frameworks is problem gamblers with comorbid depression and/or anxiety (Milosevic & Ledgerwood, 2010). Of course, comorbidity is a common feature of problem gambling, with estimates suggesting the vast majority of problem gamblers (up to 96%) have at least one other concurrent mental health disorder (Kessler et al., 2008), but those with depression and anxiety disorders seem to form a separate class. One of the most popular models, the pathways model, categorized these gamblers as the "emotionally vulnerable" subtype, who seek out gambling as a form of escape (Blaszczynski & Nower, 2002).

Empirical studies support a link between depression and disordered gambling. Studies using fMRI have shown that people with depression experience hyperactivity in reward-related brain areas during anticipation of reward and hypoactivity to outcomes (Dichter, Kozink, McClernon, & Smoski, 2012), as well as blunted responses to both positive and negative feedback in a gambling task (Steele, Kumar, & Ebmeier, 2007). Problem gamblers, when tested in a monetary incentive delay task, show reduced activity during reward outcomes like people with depression, but unlike people with depression, they also show reduced activity during anticipation (Potenza, 2014). Problem gamblers with comorbid depression, when tested in a task that involved expectations of different amounts of reward, display higher reward-related activation than controls (who have scores on the South Oaks Gambling Screen of less than 2) in brain areas associated with cravings (Fauth-Buhler et al., 2014). Higher ratings of depression are correlated with experiencing

more “dark flow”, or feelings of being completely immersed while gambling, which in turn predicts higher gambling severity (Dixon et al., 2019). Surveys have also suggested that comorbid depression is associated with greater gambling severity (Hounslow et al., 2011; Quigley et al., 2015) and that people with lifetime mood disorders take longer to achieve stable abstinence (Hodgins et al., 2005).

While human studies have pointed to specific commonalities in the brain responses of gambling and depressed individuals, to learn more about the biological connection between depression and gambling, an animal model would be extremely helpful. One such potential model is the Wistar-Kyoto (WKY) rat. WKY rats were originally bred as the normotensive control to the spontaneously hypertensive (SH) rat, but have since gained traction as an animal model of depression, as these rats exhibit high levels of depressive- and anxiety-like behaviours in forced swim, open field and defensive burying tasks (Pare, 1994; Rittenhouse, Lopez-Rubalcava, Stanwood, & Lucki, 2002), and quickly develop learned helplessness and social avoidance (Nam, Clinton, Jackson, & Kerman, 2014). These studies highlight the behavioural signature of WKY rats, which is giving up early in the face of challenge. Similar to humans with treatment-resistant depression, these rats also show wide variability in their response to antidepressants, with some rats showing a strong reduction in depression-like symptoms, while others do not (Lahmame, delArco, Pazos, Yritia, & Armario, 1997; Lopez-Rubalcava & Lucki, 2000; Will, Aird, & Redei, 2003). This makes them a particularly attractive model for studying the underlying neurobiology of depression and its relationship to gambling.

In previous studies, we have found that Long Evans rats exhibit higher motivation to work when reward was delivered on a probabilistic (i.e., a “random-ratio”) schedule, akin to the underlying payout schedule used in slot machines, rather than on a deterministic (“fixed-ratio”)

schedule where a specific number of responses is needed for every reward (Laskowski, Dorchak, Ward, Christensen, & Euston, 2019). Despite their high motivation, rats on a random-ratio schedule were no more likely than rats on the fixed-ratio schedule to develop compulsive tendencies on the task, even after two months of daily training. For example, when reward was paired with aversive foot-shock with escalating intensity, random-ratio rats quit responding just as quickly as those on a fixed-ratio schedule. Hence, our task captured the motivational features of gambling, but not the compulsion that is the hallmark of behavioral addiction. Why none of our random-ratio rats developed compulsion is unknown; one possibility is that they lacked a key vulnerability, such as impulsivity or depression, which the human literature suggests are significantly linked to addiction susceptibility (Amlung et al., 2017; Kurilla, 2021). To explore this supposition, we tested a group of rats with a depressive phenotype, namely WKY rats, on our gambling-compulsion task to see whether they would be more likely than Wistar controls to develop compulsive behaviors when exposed to prolonged training on a gambling-like reward schedule.

In our first experiment, we examined the differences between Wistar-Kyoto rats and Wistar (WIS) controls in a gambling-like task where the animal received food reward on a RR-50 schedule (i.e., reward delivered randomly with an average response ratio of 50). After four weeks of training, the animals were subjected to a series of addiction tests commonly used to assess addiction in animal models, specifically, persistent responding in the face of countervailing cues, increasing work requirements, and progressively increasing negative consequences (Belin et al., 2008; Deroche-Gamonet, Belin, & Piazza, 2004; Laskowski et al., 2019). Given the strong link between depression and problem gambling, we hypothesized that WKY rats, being more depression-prone, would display more compulsive reward-seeking behaviours than WIS rats.

Indeed, we observed that WKY rats were far more likely to persist in responding despite countervailing cues and increasing negative consequences (i.e., foot shock). In our second experiment, we sought to test whether the compulsion observed in our WKY rats was specific to the gambling task as opposed to a general characteristic of the task, such as repetitive responding. Hence, we re-ran the first experiment but added a control group trained on a fixed-ratio (reward delivered consistently after a fixed amount of lever presses) schedule. We predicted that on this task, which is obviously not gambling-like, the WKY rats would show notably lower rates of perseveration and would not differ from their WIS controls. This would establish a strong rodent model for the depression-linked pathway to gambling addiction.

Methods

Subjects

Subjects were half male Wistar-Han IGS rats and half male Wistar-Kyoto rats (Experiment 1: n=24, Experiment 2: n=36; Charles River Laboratories, Kingston, ON). In Experiment 1, rats were shipped at ~24 days old, but these animals were not tested in this paradigm until ~24 weeks of age and were not experimentally-naïve: from post-natal day 32-38, they were tested daily in a play paradigm where they were introduced into a small test chamber, held for two minutes, then allowed to play with a partner for 10 minutes before returning to their home cage; these rats were socially isolated during this period, except for the 10 minutes of social play (Burke et al., 2021). In Experiment 2, rats were shipped at 10 weeks old and began testing at ~15 weeks of age and had no prior experimental experience. In both experiments, one rat had to be excluded from analysis (Experiment 1: WISRR, Experiment 2: WKYFR) for chewing on the lever, instead of pressing with the forepaws. All experiments were performed in accordance with the Canadian Council of Animal Care and the University of Lethbridge Animal Welfare Committee.

All animals were pair-housed in a temperature-controlled room, kept under a 12-hour reverse light cycle, and were at least 100 days old at the beginning of food restriction. Animals were food restricted to ~85% of their free-feeding body weight over a period of no more than two weeks and maintained at this weight for the duration of the experiment. Supplemental rat chow was provided daily to maintain weight targets, at least 10 minutes after testing. Water was available ad libitum.

Apparatus

Testing occurred in standard five-hole operant chambers, within ventilated sound-attenuating cabinets (Med Associates Inc., Fairfax, VT) that each had a small fan (Mechatronics, F8025E24B) which provided ventilation, as well as an auditory mask. On one wall, reward (Rodent Purified Dustless Precision Pellets, F0021, 45 mg; Bio-Serv, Flemington, NJ) was dispensed into a tray with an infrared beam. In Experiment 1, to the left of the tray was a non-retractable lever with an internal cue light, and on the right, water was available ad libitum. In Experiment 2, the chambers were outfitted with retractable levers on both sides of the tray, but only the left lever was used, with a green cue light above. In both experiments, the light associated with the lever was illuminated when the lever was "active", meaning that pressing the lever would lead to a reward, and extinguished once the lever was depressed. The opposite wall contained five nose-poke holes with lights. In Experiment 2, water was provided ad libitum above the nose-poke holes. Two houselights (blue and yellow) could illuminate the chamber. In both experiments, a standard Microsoft Windows computer operated the hardware via software written in ABET II (Lafayette Instruments, Lafayette, MA) and matching interface hardware (ABET 2G starter interface and expansion interfaces). Shockers from Lafayette Instruments (model HSCK100AP) delivered scrambled shock with a bipolar waveform and 12% duty cycle. The scrambler has a cycle time of

75 millisecond to distribute shock to all eight grid pairs, with each grid pair receiving a bipolar pulse of 8.3 milliseconds.

Elevated Plus Maze/Open Field Test

The animals were tested for anxiety-like behaviour using the elevated plus maze and video was scored by hand. Locomotor activity was assessed using an open field paradigm, where the animal explored a novel environment for 2 hours while its activity was monitored by infrared beams that lined the perimeter of the environment. Data was collected using the VersaMax Legacy Open Field apparatus (OmniTech Electronics Inc., Columbus, OH).

Plantar Test/Von Frey Test

In Experiment 2, we incorporated pain tolerance testing to rule out any strain differences in sensitivity to painful stimuli. We chose to use the plantar test and the Von Frey test because they do not require the use of shock, thus avoiding a confound with our experimental test. Animals were shuffled and given pseudonyms during pain tolerance so that the experimenter was blind to each rats' strain and assigned test condition.

In the plantar test, an infrared heat source is focused on the center of the hind paw and the latency to produce a pain response (i.e.: shaking the hind paw, biting) is measured (Hargreaves, Dubner, Brown, Flores, & Joris, 1988). This test was conducted using a specially designed Plantar Test apparatus, also known as a Hargreaves Apparatus (Ugo Basile SRL, Gemonio, Italy). The animals were habituated to the environment for 10 minutes the day before testing. On the day of testing, the animals were given 5 minutes to explore the environment before testing began. Alternating hind paws were tested at 75% intensity every 3 minutes for 3-5 repetitions, as some animals would move during testing (Dirig, Salami, Rathbun, Ozaki, & Yaksh, 1997). This allowed

for a 6-minute delay between repetitions on each paw, which ensured that the temperature of the skin had returned to baseline between measures. The heat source would automatically shut off after 20 seconds to prevent tissue damage. The first three successful measures (where the animal did not move randomly) collected from each animal were averaged and taken as their final measure (withdrawal latency).

The Von Frey test is conducted by poking the middle of the hind paw with filaments that require increasing amounts of force to bend (Deuis, Dvorakova, & Vetter, 2017). The animals were habituated to the new environment for 5 minutes the day before testing, and on the day of testing, they were given another 5 minutes to explore the environment before testing began. The animals were placed in a plexiglass chamber with a grid floor, and each hind paw was poked 5 times with a filament. If the animal did not respond on 60% of the trials, the next stiffer filament was used. Once the animal responded (i.e.: lifted paw, paw shake) to a filament 60% of the time, testing ceased, and this point was determined to be their mechanical nociceptive threshold. This test was conducted using filaments from the Touch Test Sensory Evaluator kit (Stoelting Co., Wood Dale, IL).

Gambling Task - Training

Animals were tested daily, using methods identical to Laskowski et al. (2019). One day before training, all animals received a small number of pellets in their home cages to create familiarity with the novel food. In the first stage, animals were habituated to the chamber, and pellets were delivered non-contingently over a 30-minute period. This stage was a single session. In this session and all subsequent sessions, the tray light was illuminated whenever pellets were delivered, and it remained on until the rat stuck its head into the tray to retrieve the pellets. When the pellets were dispensed, the lights within the nose-poke holes would flash three times: the first,

third and fifth holes illuminated on the first and third flash; the second and fourth holes illuminated on the second flash. In the second stage, animals were trained to press the lit lever to deliver a single pellet and were advanced once they received 100 rewards within 30 minutes (typically one to six sessions). Any time reward was available, the blue house light was illuminated. In the third stage, they were trained to associate reward unavailability with the yellow house light (typically one to seven sessions). Periodically, the blue house light was extinguished and replaced by the yellow house light. Initially, the yellow light stayed on for one minute. Once rats had reduced responding by 50% during the presentation of the yellow house light, the duration was increased. This occurred over three sessions, until the rats reach a cued-no reward period lasting 10 minutes.

Animals then completed a walk-up in ratio requirements. Every two sessions, the ratio requirement increased (5, 10, 15, 25 lever presses) until they reached the final ratio requirement. In Experiment 1, all animals were ultimately tested on an RR-50 schedule, each session required 4950 lever presses and included 99 rewards (of three pellets) delivered pseudo-randomly. In Experiment 2, groups were determined by rank ordering rats by average response rates during the final stage of training, and then half of the rats of each strain were tested on an RR-40 schedule, half on an FR-40 schedule, for a total of 3960 lever presses per session. We chose to lower the ratio requirement for Experiment 2 because the response rates of some WKY rats in Experiment 1 were extremely low and we were concerned that these low-responding individuals, if confronted with a less-motivating FR-50 schedule, might actually just stop. We used a ratio requirement of 40 because previous research has shown that this value is still sufficient to induce robust schedule-related differences in post-reinforcement pause duration (Mazur, 1983).

Gambling Task – Testing

Once the animals completed the walk-up, they were tested for four weeks. For the RR schedule, we rotated daily through seven computer-generated pseudo-random RR schedules that delivered the same total amount of reward over the same total amount of lever presses as the FR schedule. The schedules were pseudo-random as we imposed the constraint that no schedule could have more than four times the ratio requirement between rewards (200 lever presses for Experiment 1, 160 lever presses for Experiment 2) to ensure the rats did not lose interest. Each session concluded when the animal received all 99 rewards or 155 minutes elapsed. At 33 and 66 rewards, the house lights changed from blue to yellow and a 10-minute cued-no reward period began. Animals that did not obtain 99 rewards each day were run for extra days to ensure that all animals received the same amount of total reward before addiction tests.

Tests for Addiction-like Behaviour

The progressive ratio task is a one-day task used to measure motivation, in the form of persistent responding despite increasing work requirements. In this task, the number of responses required to achieve the xth reward (PR) was computed using the function $PR(x) = \text{round}(r * ((x - 3)^2/(104)^2))$, where r = ratio value at 100 rewards, set to 2000, with x ranging from 1 to 120. The session continued for 5 hours or until the rat did not obtain a reward within 1 hour.

The progressive aversion task is a one-day task that measures compulsive reward-seeking behaviour despite increasingly negative consequences. The rats received reward on an FR-10 schedule. On the 8th and 9th lever press, the lever light would flash at 6.67 Hz to signal that the next lever press would result in shock. The 9th lever press resulted in a foot shock and the 10^{th} foot shock resulted in reward and foot shock. The shock was increased by 0.04 mA every three rewards, from 0.04 mA to 1.24 mA. The task continued for 5 hours or until the animal did not obtain reward within 30 minutes.

The reinstatement task looks at the animals' propensity to respond after a period of abstinence. The rats underwent a 5-day rest period, where they were still on food restriction but given extra lab chow to maintain their target body weights, and then responding was measured over two sessions. Each session began with a 90-minute extinction period, and then cue lights associated with reward (blue house light, lever light) were activated for the remainder of the session. During the first session, at 90 minutes and every 30 minutes after, the tray light turned on and the pellet dispenser was activated but did not deliver reward inside the chamber; instead, the pellet dispenser tube was disconnected from the tray and the pellets were dropped into a cup outside of the chamber so that the animal could still hear the pellet dropping. The tray light was extinguished when the rat poked its head into the tray. The second session was the same, except reward was delivered. One pellet was delivered at 90 minutes and the total pellets delivered subsequently doubled each time (1, 2, 4, 8). Each session lasted 210 minutes Response rate data was collected from the last 30 minutes of the second session.

Statistical Analysis

Statistical analyses were performed using MATLAB (The Mathworks, Natick, MA) and IBM SPSS Statistics (IBM, Armonk, NY). In both experiments, post-reinforcement pause, cued-no reward period, progressive aversion and reinstatement data was logarithmically transformed to correct for skewness. When Mauchly's Test of Sphericity was significant, the Greenhouse-Geisser correction was reported.

Results

Elevated Plus Maze and Open Field Testing

Two standard measures of anxiety, the elevated plus maze and open field tests, were obtained for all animals. In Experiment 1, one-way ANOVAs revealed that the WKY rats spent significantly less time in the open arms of the elevated plus maze ($F_{(1,21)} = 126.235$, $p < 0.001$; WKY: mean = 13.4167, standard error of the mean (SEM) = 3.77483; WIS: mean = 89.1818, SEM = 5.72049) and traveled less distance in the open field test ($F_{(1,21)} = 24.014$, $p < 0.001$; WKY: mean = 880.3333, SEM = 100.38014; WIS: mean = 3889.2727, SEM = 633.22018). There was no difference in thigmotaxis between strains ($F_{(1,21)} = 1.236$, $p = 0.279$; WKY: mean = 3429.7167, SEM = 74.11858; WIS: mean = 3230.2727, SEM = 169.31964). In Experiment 2, we found no difference between the strains in the time spent in the open arms ($F_{(1,33)} = 0.898$, $p = 0.350$; WKY: mean = 34.5882, SEM = 5.05562; WIS: mean = 28.0000, SEM = 4.77877) or in thigmotaxis during the open field task ($F_{(1,33)} = 2.443$, $p = 0.128$; WKY: mean = 7025.8824, SEM = 25.33425; WIS: mean = 6937.3167, SEM = 49.56509), but the WKY rats continued to travel less distance in the open field ($F_{(1,33)} = 37.211$, $p < 0.001$; WKY: mean = 1379.9412, SEM = 166.36570; WIS: mean = 4211.1667, SEM = 422.49797).

Gambling Task – Response Rates and Post-Reinforcement Pausing

During the four weeks of testing on the gambling task, the rats in Experiment 1 were given daily experience obtaining reward on either an RR-50 or FR-50 schedule, and the rats in Experiment 2 obtained reward on an RR-40 or FR-40 schedule. Response rates were calculated by averaging responses per second for each animal across each week of testing. In Experiment 1, a two-way mixed ANOVA, with the between subjects factor strain and the repeated factor week, showed that all rats became faster over time ($F_{(1.824,38.313)} = 15.879$, $p < 0.001$; Greenhouse-Geisser corrected), however, the WKY rats had significantly slower response rates ($F_{(1,21)} = 4.355$, $p = 0.049$). In Experiment 2, we replicated these findings using a three-way mixed ANOVA with

between factors strain and schedule, and the repeated factor week. All animals responded faster over time ($F_{(1.919,59.500)} = 34.675$, $p < 0.001$; Greenhouse-Geisser corrected) and the WKY rats were significantly slower ($F_{(1,31)} = 17.756$, $p < 0.001$). Surprisingly, there was no difference in response rates between schedules ($F_{(1,31)} = 0.477$, $p = 0.495$) and there was no significant three-way interaction ($F_{(1.919,59.500)} = 0.615$, $p = 0.538$; Greenhouse-Geisser corrected).

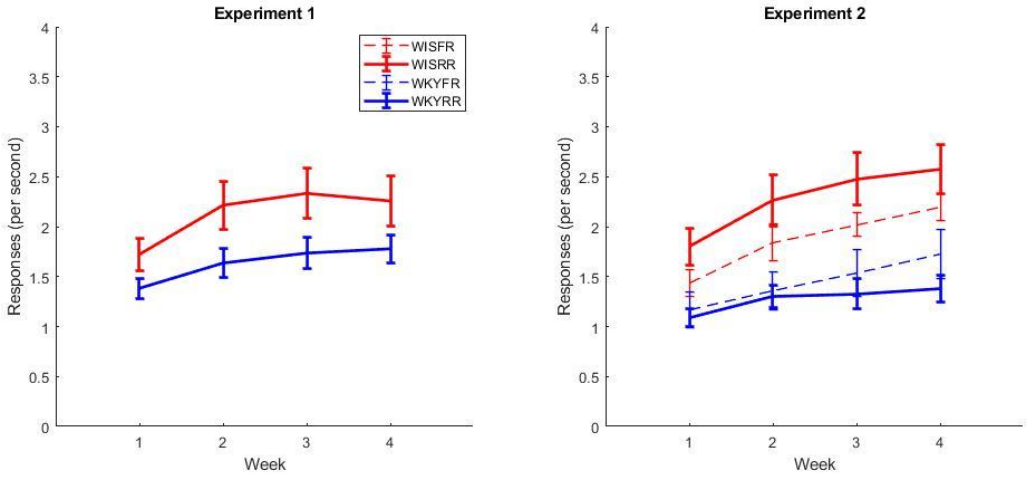

Figure 1: Average response rates over four weeks of testing Wistar and Wistar-Kyoto rats on random-ratio or fixed-ratio schedules of reinforcement. Post-reinforcement pauses and responses made during the cued-no reward were excluded. The blue lines represent the WKY rats, while the red lines represent the WIS rats. Solid lines indicate that the animal was trained on an RR schedule, while dashed lines demarcate animals trained on an FR schedule. Error bars show SEM.

Post-reinforcement pause (PRP) duration was measured from the last time the rat removed its head from the food tray until its next lever press. The PRP typically gets longer as individuals lose motivation (Mazur, 1983). In Experiment 1, a two-way mixed ANOVA, with the between subjects factor strain, showed that WKY rats had significantly longer PRPs ($F_{(1,21)} = 16.857$, $p = 0.001$). There was a significant week x strain interaction, indicative of an increase in the WKY

rats' PRP duration over time ($F_{(2.199,46.174)} = 3.787$, $p = 0.026$; Greenhouse-Geisser corrected). In Experiment 2, a three-way mixed ANOVA, with between factors strain and schedule, and the repeated factor week, revealed that the WKY rats continued to pause for longer ($F_{(1,31)} = 6.589$, $p = 0.015$) and the rats on the RR schedule were much quicker to initiate the next trial ($F_{(1,31)} = 19.757$, $p < 0.001$). There was no significant week x strain interaction ($F_{(1.759,54.517)} = 1.070$, $p = 0.343$; Greenhouse-Geisser corrected) but there was a significant week x schedule interaction ($F_{(1.759,54.517)} = 7.591$, $p = 0.002$; Greenhouse-Geisser corrected) as the FR rats' PRPs shortened over time. There was no significant three-way interaction ($F_{(1.769,54.517)} = 0.483$, $p = 0.595$; Greenhouse-Geisser corrected). These findings show that both strain and schedule contribute to PRP duration, with WKY rats and those on a FR schedule having longer PRPs.

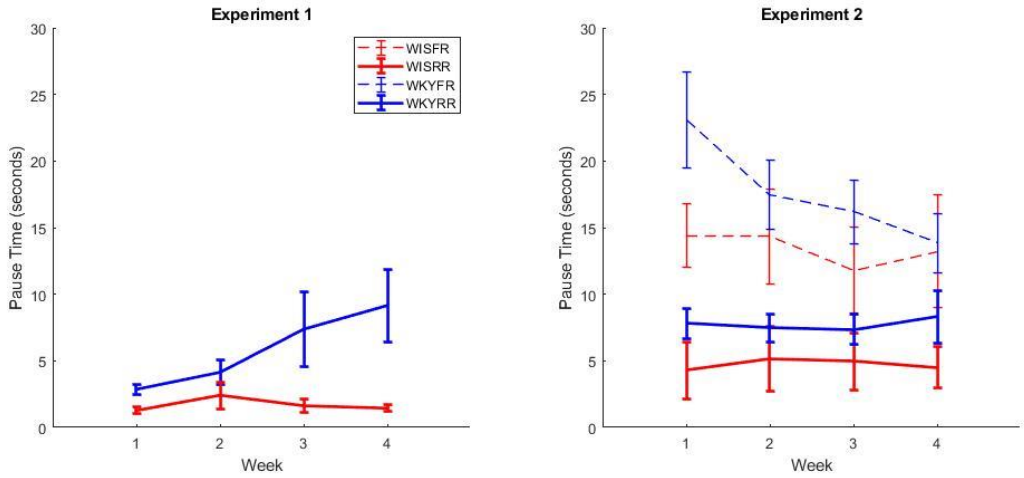

Figure 2: Effects of strain and reinforcement schedule on post-reinforcement pause duration over four weeks of testing. The blue lines represent the WKY rats, while the red lines represent the WIS rats. Solid lines indicate that the animal was trained on an RR schedule, while dashed lines signify that the animals were trained on an FR schedule. Error bars show SEM.

Gambling Task – Cued-No Reward Period

Response rates during the period when a cue indicated that reward was not available (cued-no reward period) were expressed as a ratio of the response rate when cues indicated that reward was available, referred to here as a normalized response rate. In Experiment 1, a two-way mixed ANOVA, with the between subjects factor strain, showed that the WKY rats responded significantly more during the cued-no reward period ($F_{(1,21)}$ = 11.141, p = 0.003) and that all rats reduced their responding over time ($F_{(3,63)}$ = 28.424, p < 0.001). In Experiment 2, a three-way mixed ANOVA, with between factors strain and schedule, and the repeated factor week, showed that the WKY rats continued to respond significantly more during the cued-no reward period ($F_{(1,31)}$ = 16.977, p < 0.001) but there was no schedule effect ($F_{(1,31)}$ = 1.716, p = 0.200). Again, all of the animals responded significantly less during the cued-no reward period over time ($F_{(2.117,65.614)}$ = 31.031, p < 0.001; Greenhouse-Geisser corrected). There was no significant week x strain ($F_{(2.117,65.614)}$ = 2.409, p = 0.095; Greenhouse-Geisser corrected), week x schedule ($F_{(2.117,65.614)}$ = 0.534, p = 0.599; Greenhouse-Geisser corrected), or three-way interaction ($F_{(2.117,65.614)}$ = 0.989, p = 0.381; Greenhouse-Geisser corrected).

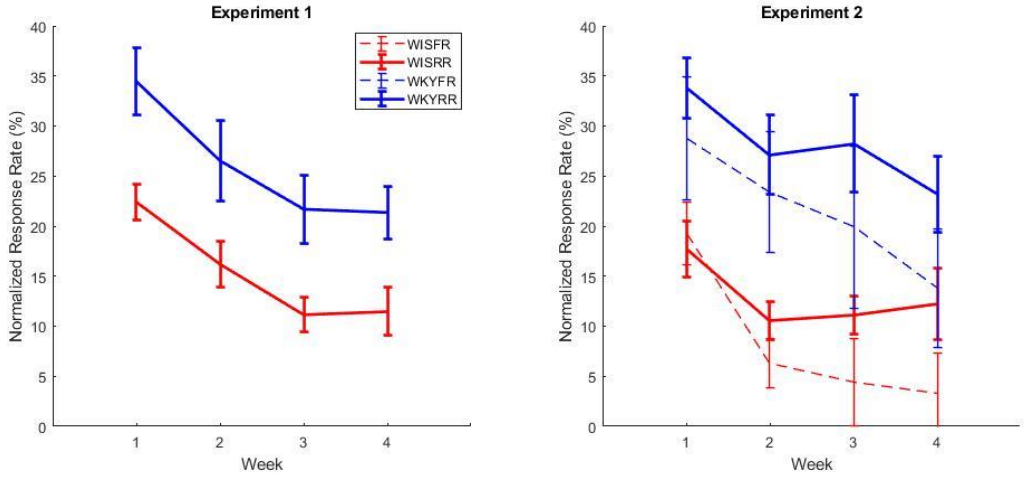

Figure 3: Normalized response rate during the cued-no reward period over four weeks. Response rate was expressed as a percentage of the response rates during the periods where reward was available. The blue lines represent the WKY rats, while the red lines represent the WIS rats. Solid lines indicate that the animal was trained on an RR schedule, while dashed lines mean animals were trained on an FR schedule. Error bars show SEM.

Progressive Ratio Task

Performance on the progressive ratio task was measured by the "breakpoint", the maximum ratio that rats achieved before they stopped responding. In Experiment 1, a one-way ANOVA found no significant difference between strains ($F_{(1,21)} = 1.247$, $p = 0.277$) in their breakpoints. In Experiment 2, a two-way ANOVA, with the between subjects factor strain, found that WKY rats had significantly lower breakpoints ($F_{(1,30)} = 12.878$, $p = 0.001$) but there was no effect of schedule ($F_{(1,30)} = 1.338$, $p = 0.257$). In Experiment 2, one animal had to be excluded due to technical issues with the chamber (WKYFR).

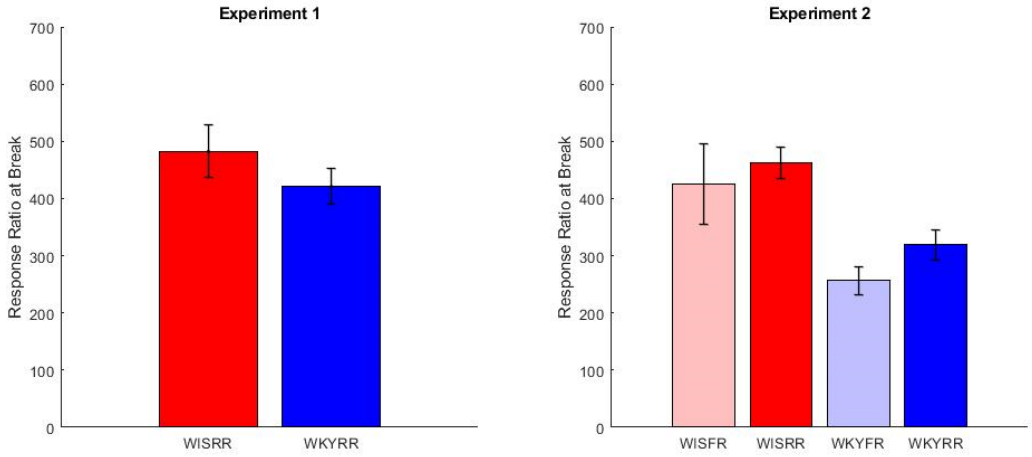

Figure 4: Effects of strain and reinforcement schedule on the breakpoint of reward-seeking behaviour when faced with increasing ratio requirements. The final ratio requirement that led to

relinquishing was recorded. In the first graph, the blue bar represented the WKY rats, while the red bar represented the WIS rats, and all animals were trained on an RR schedule. In the second graph, the pale-coloured bars indicate the results from the animals that were trained on an FR schedule. Error bars show SEM.

Progressive Aversion Task

In the progressive aversion task, reward delivery was paired with a mild foot shock of gradually increasing intensity. As with the progressive ratio task, performance on this task was assessed via the breakpoint, except the breakpoint in this case was maximum current, instead of maximum ratio. WKY rats had significantly higher breakpoints than WIS rats, in Experiment 1, as determined by a one-way ANOVA ($F_{(1,21)} = 18.586$, $p < 0.001$). In Experiment 2, a two-way ANOVA, with the between subject factor strain, found that the WKY rats continued to have higher breakpoints ($F_{(1,29)} = 8.929$, $p = 0.006$) but there was no effect of schedule ($F_{(1,29)} = 2.066$, $p = 0.161$). In Experiment 2, two animals had to be excluded due to technical issues with the chamber (both WISRR).

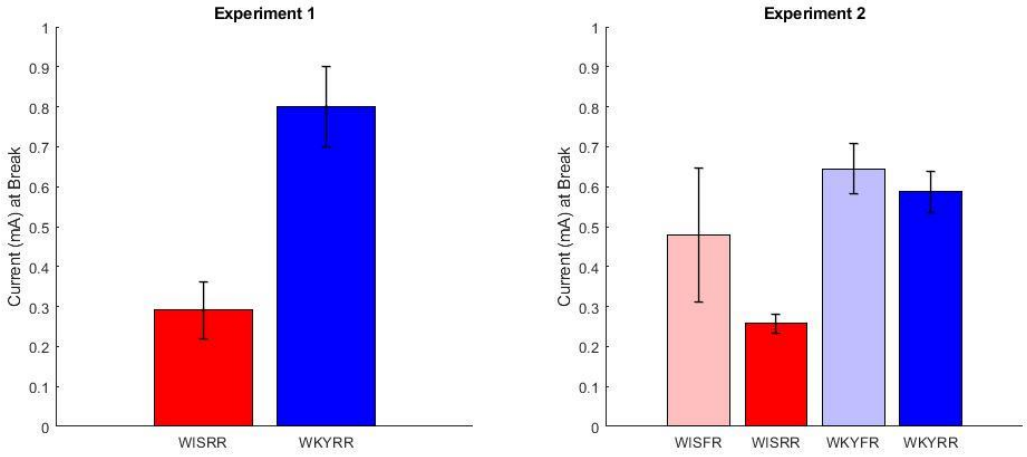

Figure 5: Effects of strain and reinforcement schedule on the breakpoint of reward-seeking behaviour when faced with increasing levels of foot shock. The final current level that deterred further responding was recorded. In the first graph, the blue bar represented the WKY rats, while the red bar represented the WIS rats, and all animals were trained on an RR schedule. In the second graph, the pale-coloured bars indicate the results from the animals that were trained on an FR schedule. Error bars show SEM.

Reinstatement Test

Our reinstatement test looked at the response rate in animals placed in the testing chamber five days after the progressive aversion task, during which time there was no subsequent training. Rats were not rewarded on the first day, but five rewards were delivered on the second day, and the normalized response rate was calculated using the last 30 minutes after the fifth reward was dispensed. In Experiment 1, a one-way ANOVA found no significant difference between strains ($F_{(1,19)} = 0.847$, $p = 0.369$). In Experiment 2, a two-way ANOVA, with the between subjects factor strain, found no significant differences between strains ($F_{(1,31)} = 0.000$, $p = 0.983$) or schedules ($F_{(1,31)} = 0.658$, $p = 0.423$). In Experiment 1, two animals (both WISRR) had to be excluded from the reinstatement task due to experimenter errors that led to the animals being fed before the task.

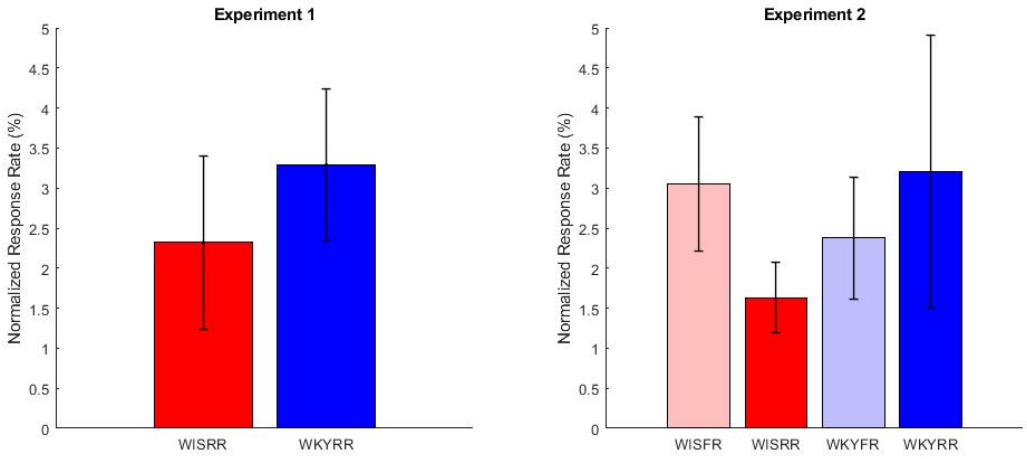

Figure 6: Normalized response rate during the reinstatement task. Response rates were expressed as a percentage of the response rate during the last week of regular testing. In the first graph, the blue bar represented the WKY rats, while the red bar represented the WIS rats, and all animals were trained on an RR schedule. In the second graph, the pale-coloured bars indicate the results from the animals that were trained on an FR schedule. Error bars show SEM.

Pain Tolerance Testing

Given the surprising tenacity of WKY rats on the progressive aversion task, we wondered whether they might have higher pain thresholds. To assess this, in Experiment 2, we included two standard tests of pain tolerance, the Von Frey test and the plantar test. A one-way ANOVA found no significant difference between strains in the plantar test ($F_{(1,34)} = 0.305$, $p = 0.585$; WKY: mean = 10.3185, SEM = 0.30507; WIS: mean = 10.5963, SEM = 0.40038) or in the Von Frey test ($F_{(1,34)} = 0.013$, $p = 0.911$; WKY: mean = 25.8889, SEM = 5.38510; WIS: mean = 26.7778, SEM = 5.81162). Curiously, there was no correlation between measures ($r = -0.140$, $n = 36$, $p = 0.416$).

Discussion

WKY rats had slower response rates, longer post-reinforcement pauses, and lower breakpoints in the progressive ratio task. Taken together, this indicates low motivation to obtain reward. In fact, the WKY rats are the only strain that we have looked at that does not show faster response rates on the RR schedule (Laskowski et al., 2019). Despite this apparently lower motivation, the WKY rats responded more during the cued-no reward period and the progressive aversion task, demonstrating persistent reward-seeking behaviour in the face of countervailing cues and increased effort. In other words, at least on these two measures, these rats were more likely than WIS rats to acquire compulsive reward-seeking after prolonged training. The only measure in which the WKY rats showed significantly different responses due to schedule was in post-reinforcement pause (PRP), where their pauses were longer on the FR than RR schedule; this was no surprise as PRPs have been shown to be one of the hallmark differences between RR and FR schedules (Mazur, 1983). PRPs were also longer in WKY rats than WIS rats, again consistent with an overall lower level of motivation in the WKY rats. The fact that WKY rats were just as likely to show compulsive reward-seeking after FR schedules as RR schedules suggests that they are not a good gambling model. However, when compared to the WIS rats, they appear to provide a strong model of compulsive behaviour.

Depressed gamblers often seek out gambling activity as a form of escape (MacLaren et al., 2015). Entering a state of "dark flow" may capture the chronically wandering mind and provide temporary relief from rumination (Dixon et al., 2019). One limitation of this model, and animal models of depression in general, is that animals probably do not spend time ruminating on negative thoughts like humans do; hence, it is unlikely that the rats were pressing the lever to achieve some form of escape. However, our research shows that "depressed" rats are more vulnerable to

developing compulsion after repetitive activities, so at least at a behavioural level, there appear to be some parallels between rats and humans.

The lack of correlation in the pain tolerance between our Von Frey test and plantar test measures is troubling, but this is likely because the Von Frey test is better suited to measure mechanoreception than pain. In the Von Frey test, not all responses were pain responses (i.e.: paw shake, paw bite), and some animals became aware of the experimenter and would watch their hand through the grid floor, suggesting that a lot of responses could be due to sensation. We combatted these false positives by increasing the threshold of responding to 60%. The plantar test, in contrast, involves rapid heating of the foot via laser and is likely a more valid measure of pain. Regardless, the lack of significant strain difference in either task argues strongly that the behaviour differences between WKY and WIS rats is not due to differences in pain perception.

WKY rats have been tested in other addiction paradigms with varying results. Compared to spontaneously hypertensive (SH) rats, WKY rats consume more ethanol when given free access (Soeters, Howells, & Russell, 2008), and when compared to Wistars, are quick to develop sugar binging-like behaviour, although the females seem more susceptible to this (Papacostas-Quintanilla, Ortiz-Ortega, & Lopez-Rubalcava, 2017). On the other hand, a study comparing nicotine administration in six inbred rat strains and six F1 hybrids (used to examine the extent to which nicotine intake was genetic and could be predicted by the parental strains) categorized WKY rats in the low self-administration category compared to Lewis rats and SH rats (H. Chen, Hiler, Tolley, Matta, & Sharp, 2012). Similar to Sprague Dawley rats, WKY rats do not show a conditioned place preference to methamphetamine like the SH rats do (Womersley et al., 2016), but unlike Sprague Dawley rats, they do show a conditioned place preference for cocaine when given more conditioning trials (Dennis, Beck, Bobzean, Dougall, & Perrotti, 2012). WKY rats are

slower to reach criteria for acquisition of cocaine self-administration compared to SH rats, but unlike SH rats, their cocaine self-administration can be potentiated by atomoxetine treatment during adolescence (Somkuwar, Jordan, Kantak, & Dwoskin, 2013). Although they self-administer less cocaine (Harvey, Sen, Deaciuc, Dwoskin, & Kantak, 2011; Jastrzebska et al., 2015; Jordan, Harvey, Baskin, Dwoskin, & Kantak, 2014), they have similar rates of extinction to SH rats, both strains taking longer than Wistars (Jordan et al., 2014). However, WKY rats (like WIS rats) appear to be less motivated than SH rats to obtain cocaine, as assessed by a progressive ratio schedule (Harvey et al., 2011). Repeated forced swim stress exposure did not enhance responding for cocaine in WKY rats, but it did in Wistars (Groblewski, Zietz, Willuhn, Phillips, & Chavkin, 2015), which may be a saturation effect as the WKY rats were already "depressed". Hence, with the possible exception of ethanol, WKY rats do not appear to be a particularly good model of substance abuse. One key difference between these previous studies and our own is the duration of training. It could be that addiction-like behaviour only emerges in WKY rats after prolonged, repetitive training; or that they are only addicted to depressants, and are not as vulnerable to stimulants. Alternatively, it may be that WKY rats are only useful as a model of behavioural addiction, and not addiction more broadly.

It would be interesting to subject normal rats, such as WIS rats, to a chronic mild stress paradigm, which has been shown to induce depression-like symptoms (Willner, 2017). One study found that in a learned helplessness paradigm, baseline rGT performance did not predict learned helplessness, but the ensuing learned helplessness protocol increased poor decision-making in subsequent rGT sessions, suggesting that something about the learned helplessness paradigm decreased advantageous decision-making (Nobrega et al., 2016). Given the speed at which WKY rats develop learned helplessness, and that stress is intricately linked to depression, we would

likely see similar results (i.e., compulsive responding despite countervailing cues, increased work requirements and increased punishment) from the chronic mild stress animals as what we have seen in the WKY rats. It is also possible that a stressed normal rat might be more sensitive to a gambling schedule, because these animals are unlikely to have pre-existing motivational deficits to the same extent that WKY rats have. Hence, they may show the motivational aspects that the WKY model lacks.

Animal models have played a key role in our understanding of the neural mechanisms underlying addiction. On a genetic level, the roles of each opioid receptor class in the addiction cycle were established using transgenic mice with knockouts for various opioid receptors (Lutz & Kieffer, 2013) and increased transcriptional activity, examined through immunohistology, in the nucleus accumbens after repeated cocaine exposure was found to be the result of amplified acetylation of H3 histones in this region (Nestler, 2014). On a cellular level, hypoactive electrophysiological changes in specific neuron populations have been reported in rats who self-administer cocaine (B. T. Chen et al., 2013). The influence of various brain regions can also be examined. For example, repeated optogenetic self-stimulation in reward processing areas, such as the ventral tegmental area and projections from the basolateral amygdala to the nucleus accumbens, has been reported (Tye & Deisseroth, 2012) and is further evidence to implicate these regions in the development and maintenance of addiction.

While some researchers may argue about whether something as complex as gambling addiction can truly be modelled in animals, there are many exciting findings that suggest it is possible to study aspects of the gambling experience in other species. The occurrence of single nucleotide polymorphisms, or single substitutions in a genetic sequence, in the DRD3 and CAMK2D genes has been correlated with problem gambling in humans and decreased rodent

gambling task (rGT) performance in rats (Lobo et al., 2015). Both genes play a role in dopamine transmission. Dopamine agonist therapy, a treatment for Parkinson's disease, is linked to high rates (nearly 18% in high affinity D3 agonists) of developing behavioural issues after starting medication, like problem gambling or compulsive shopping (Weintraub et al., 2010). Selective dopamine agonists, like ropinirole and pramipexole, have been studied in rats with mixed results on compulsive reward-seeking: some studies showing increased compulsivity (Cocker, Lin, Tremblay, Kaur, & Winstanley, 2019) and others showing increased motivation without developing into full-blown compulsion (Laskowski et al., 2019). There are many possibilities in the realm of animal studies, and their development is important as we try to learn more about the neural underpinnings of gambling addiction.

Conclusion

Despite showing lower motivation to obtain reward, WKY rats showed more compulsive reward-seeking behaviour, specifically, persistent responding during the cued-no reward period and the progressive aversion task. This behaviour was not a result of the schedule of reinforcement but suggests that WKY rats may be an interesting strain of rat to use to model other forms of addiction.

CHAPTER 3: IMPULSIVITY

Introduction

Different schedules of reinforcement each produce a distinct behavioural profile. On one hand, there are fixed-ratio (FR) schedules, where a person (or animal) is rewarded consistently after a certain amount of work is performed; on the other hand, there are random-ratio (RR) schedules that reward the participant probabilistically, like the payout structure of a slot machine. FR schedules result in slower response rates and longer post-reinforcement pauses (the latency to initiate the next trial after being rewarded) than what is produced by RR schedules (Ferster & Skinner, 1957; Mazur, 1983; Schlinger, Derenne, & Baron, 2008). Together, these characteristic behaviours suggest that the RR schedule is much more motivating than the FR schedule. But why is that?

One theory is that the appeal of RR schedules is related to delay discounting (Madden et al., 2007). Delay discounting, a measure of choice impulsivity, is phenomenon where the subjective value of a reward decreases over time. The subjective value has been shown empirically to follow a hyperbolic function: $A / (1 + kD)$; where A is the actual value of the reward, k is the discounting parameter, and D is the delay (Ainslie, 1975; Mazur, 1984, 1986). The k value determines the slope; those with higher k values have steeper delay discounting curves, as they discount future rewards more heavily (Winstanley, 2011). This includes problem gamblers, who discount rewards at much faster rates than healthy controls (Petry, 2001).

The delays between rewards on random-ratio schedules are skewed towards shorter values, as seen in the histogram in Figure 7, which are necessary to compensate for the rewards that take longer to obtain, and are a natural consequence of how the RR schedule functions (Haw, 2008).

This means that when the subjective value is summed across trials, people with higher k values will be drawn to the RR schedule, as the subjective value of the few immediate rewards outweighs that of the consistent but delayed rewards (Madden et al., 2007). This idea is illustrated in Figure 7, where the subjective value, indicated by the red line, is seen decreasing hyperbolically over time. If you consider that the overall value of a schedule is the sum of all rewards obtained weighted by their delay discounting, it becomes apparent that the subjective value of an FR-50 schedule (whose fixed delay is indicated by the arrow) would be much lower than that of an RR-50 schedule, despite both schedules requiring the same amount of work to obtain the same amount of reward.

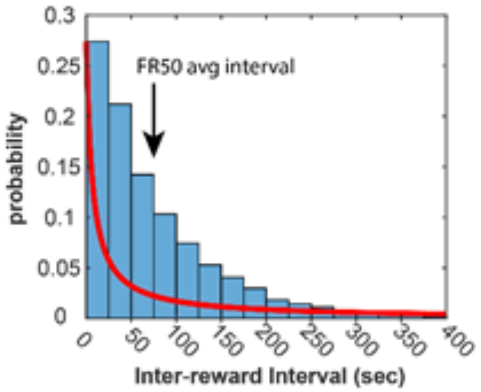

Figure 7: A histogram of time intervals between rewards delivered on an RR-50 schedule, assuming each response took approximately 1.5 seconds. The delay discounting curve, representing the subjective value of a reward as the delay increases over time, for an animal with a k value of 0.15 is overlaid in red.

There is mounting evidence supporting impulsivity's role in gambling addiction: impulsivity (MacLaren et al., 2012) and the steepness of delay discounting curves (Punia & Balodis, 2019) predict gambling severity; delay discounting is significantly correlated with susceptibility to cognitive distortions (Michalczuk et al., 2011); and while there is a significant

relationship between performance on the Eysenck Impulsivity Scale and delay discounting, the South Oaks Gambling Screen, a measure of gambling severity, is 1.4 times better at predicting delay discounting behaviour in problem gamblers (Alessi & Petry, 2003), suggesting that this specific form of impulsivity is highly linked to gambling pathology. Could the fact that gambling-like schedules of reinforcement are skewed towards immediate rewards explain why those who are impulsive are more vulnerable to gambling addiction? We believe that after measuring an animal's impulsivity in a delay discounting task, we will be able to predict its preference for gambling-like schedules of reinforcement. Towards this end, we developed a novel task to assess an animal's preference for an RR over an FR schedule, which we refer to as the "schedule preference task".

The schedule preference task gives the animals a choice between two levers: one offers reward on an RR-50 schedule and one offers reward on an FR-50 schedule; the ratio values dynamically adjust until an animal is choosing equally between the two, from which we can surmise the degree to which the animal prefers receiving random rewards. The following paper outlines the first two pilot experiments as we developed the schedule preference task, establishing just how much more motivating the random-ratio schedule is. Further, we report a test of whether more impulsive rats would have a stronger preference for RR schedules.

As mentioned before, the delay discounting task – where a choice is made between a small, immediate reward and a large, delayed reward – is often used as a measure of choice impulsivity and has great translational potential from rodents to humans. We sought to correlate performance between the delay discounting task and our schedule preference task. If, as predicted, RR schedules are more attractive to impulsive animals, it would provide evidence for Madden's (2007) theory that delay discounting makes RR schedules more enticing.

Methods

Subjects

Subjects were male Long Evans rats (Experiment 1: n=12, Experiment 2: n=12; Charles River Laboratories, Kingston, ON). In Experiment 1, rats were shipped at 11 weeks old; in Experiment 2, rats were shipped at 8 weeks old. In Experiment 2, both shipping and inventory availability were negatively impacted due to the COVID-19 pandemic; as such, these animals were subjected to longer shipping times and were younger on arrival. All experiments were performed in accordance with the Canadian Council of Animal Care and the University of Lethbridge Animal Welfare Committee.

All animals were housed in sets of three in a temperature-controlled room and kept under a 12-hour reverse light cycle. The rats were aged in house until 100 days old, at which time they were food restricted to ~85% of their free-feeding body weight over a period of no more than two weeks. The animals were maintained at this weight for the duration of the experiment. Supplemental rat chow was provided daily to maintain weight targets, at least 10 minutes after testing. Water was available ad libitum. Animals were tested daily. One day before testing, all animals received a small number of pellets in their home cages to create familiarity with the novel food.

Apparatus

Testing occurred in standard five-hole operant chambers, within ventilated sound-attenuating cabinets (Med Associates Inc., Fairfax, VT). On one wall, reward (Rodent Purified Dustless Precision Pellets, F0021, 45 mg; Bio-Serv, Flemington, NJ) was dispensed into a tray with an infrared beam. The chambers were outfitted with retractable levers on both sides of the

tray. There were green cue lights above both levers. The opposite wall contained five nose-poke holes with lights. Water was provided ad libitum above the nose-poke holes. A standard Microsoft Windows computer operated the hardware via software written in ABET II (Lafayette Instruments, Lafayette, MA) and appropriate interface hardware (ABET 2G starter interface and expansion interfaces).

Delay Discounting Task – Training

Only the animals in Experiment 2 were tested in the delay discounting task. The delay discounting task was conducted using the methods established by Mar and Robbins (2007). In the first phase, rats are trained to nose-poke into the food tray, as cued by the tray light, to extend either the left or right lever. After pressing the lever once, it retracts, and one pellet is dispensed into the tray. The rat must press the lever within 30 seconds, or it retracts and the rat receives a five second timeout. On the next trial, the opposite lever extends. The animal must receive 60 rewards in one session to advance to the next phase. The second phase is the same, except the rat must complete each step within 10 seconds of the previous step. If the rat takes more than 10 seconds at any step, or after successfully completing a trial, the rat is subjected to a 40 second timeout, or inter-trial interval, where no cues are present. Again, the rat must successfully complete 60 trials to advance to the final stage.

Delay Discounting Task – Testing

The third stage is the testing stage. This stage consists of five blocks of 12 trials: two forced trials followed by ten free trials. The rat must nose-poke to extend one (in the forced trials) or both (in the free trials) levers. One lever offers an immediate reward of one pellet, while the other lever offers a bigger reward (four pellets) after a delay. The delay increases with each block (0, 10, 20,

40, 60 seconds). The rat must nose-poke into the tray, select a lever, wait for the delay if applicable, and then receive its reward. In between each trial, the rat is subjected to a 100 second inter-trial interval before it can initiate the next trial. The animals are tested until the discounting curves are considered stable, as determined by a two-way repeated measure ANOVA of the group discounting curves, where choice ratio is the dependent variable, and 3-session block and delay are the within-subject factors. There must be no significant effect of block, and a significant effect of delay or a significant block by delay interaction, to be deemed "stable".

Schedule Preference Task – Training

This task was based on methods derived from the work of Mazur (2007) and Johnson et al. (2011). In the first stage, animals were habituated to the chamber, and pellets were delivered non-contingently over a 30-minute period. This stage was a single session. In the second stage, animals were trained to press the lever to deliver a single pellet, as cued by the green lights above the lever, and were advanced to the next stage of training once they received 100 rewards within 30 minutes. In this stage, the available lever alternated. The lever on one side retracted after being pressed and the tray light would come on until the rat retrieved the reward; the opposite lever would extend after the rat obtained its reward from the tray and the tray light would turn off. In the third stage, animals were trained to nose-poke in the middle hole on the wall opposite to the tray, as cued by a light within the hole, to make one of the levers extend. Once a lever had extended, the rat could press the lever to deliver a single pellet. As before, rats were advanced to the next stage once they received 100 rewards within 30 minutes. The second and third stages tended to last about a week. The fourth stage was a walk up, where rats were given two levers with equal average response requirements, but different schedules: one was an FR schedule and the other was an RR schedule. As before, the lever that extended alternated on each trial. The rats had to complete 100 trials, the

average number of lever presses required to acquire each reward was increased over three sessions (5, 10, 25) and instead of receiving one pellet, they now received three. The random-ratio lever was randomly assigned to either the left or right lever for each rat. It should be emphasized that, although the schedules differed, the RR lever required the same total number of lever presses as the FR lever when averaged over the session.

Schedule Preference Task – Testing

Once the rats finished training, they began running on the full task. The task included 96 trials per day, which were broken down into blocks consisting of four trials: two forced trials and two free trials. At the beginning of each block, the middle nose-poke light would illuminate, and the rat would nose-poke to extend one of the levers, turning off the nose-poke light and turning on the green cue light above the extended lever in the process. The rat would press the lever however many times were required, and then the lever would retract, and the rat would collect its reward. On the next trial, the opposite lever would extend, as it had in training. On the free choice trials, both levers extended, and the rat could choose which schedule it wanted to work for reward on. As soon as the animal selected a lever, the other lever would retract.

In Experiment 1, the RR lever delivered reward steadily on a pseudo-random RR-50 schedule, while the FR lever started as FR-50 and dynamically adjusted after each block. If the rat chose the RR lever during both free trials, the value of the FR work requirement would decrease, as to make the FR lever more attractive. If the rat chose the FR lever both times, the FR value (i.e., the number of lever presses required to obtain reward) would increase, making the reward slightly harder to obtain and thus rendering the FR lever less enticing. In Experiment 2, the RR and FR values were both adjusted at the end of each block. Now if the animal chose the RR lever both times, the FR value would still decrease, but the average RR value would also increase. The same

was true for selecting the FR lever both times: the FR value would increase, and the RR value would decrease. In Experiment 1, seven pseudo-random RR schedules were created in ABET, by programming the total number of required lever presses and the total number of rewarded lever presses, and then adjusting for any trials that exceeded 200 lever presses. The schedules were cycled through weekly, so that each day of the week was a different schedule, but so that all the animals experienced the same schedule. In Experiment 2, since the work requirement for the RR lever was now constantly adjusting too, the RR lever was programmed to deliver rewards truly randomly (i.e., the probability of obtaining reward on any lever press was a fixed value), with the exception that the work requirement for each trial could not exceed 200 lever presses, so as to maintain animals' motivation for the task. The probability of reward on any given lever press was 1/R, where the response ratio, R, was chosen to maintain the desired average response requirement, after accounting for the upper limit of 200 responses per trial. More specifically, when the work requirement for the RR lever exceeded RR-30, the current ratio requirement had to be transformed using the empirically derived formula: R = 8.012e-5*DR^3 + (-.008391)*DR^2 + 1.338*DR + -4.894; where R is the actual ratio and DR is the desired ratio. This transformation ensured that the rats continued to experience the ratio that they were intended to experience. In both experiments, the rats started on FR-50/RR-50 schedules and the final FR value at the end of the day of testing was recorded and used as the starting point for training on the next, providing continuity across days of testing. The values continued to adjust over blocks until the rat reached an equivalence point, as determined by stability criteria.

Stability criteria was adapted from similar experiments done by Mazur (2007) which looked at reward preference under fixed and adjusting delays. Each rat had to meet four criteria for the equivalence point to be considered stable: at least 20 sessions must occur before a rat can

reach criteria, the last three sessions cannot contain the highest or lowest value, the mean of the last three sessions is not the highest or lowest value, and the mean of the last three sessions is not different by more than 10% of the preceding three sessions. The mean FR value of the first session where a rat reaches criteria is its equivalence point, or the equivalent FR value, which represents the FR ratio required for the animal to choose both levers equally.

Statistical Analysis

Statistical analyses were performed using MATLAB (The Mathworks, Natick, MA) and IBM SPSS Statistics (IBM, Armonk, NY). Data from delay discounting was fit to a hyperbolic function of the form $V = A / (1 + kD)$, where A is the value of reward at zero delay, k is the delay discount factor, and D is the delay in seconds. The MATLAB function fminsearch was used to find the best fitting values of A and k for each animal.

Results

The final k values from the delay discounting task, as determined by fitting the data to a hyperbolic function from the last seven days of testing, ranged from 0.04/second to 1.86/second. The data from the rats that chose the large reward at zero delay less than 50% of the time (rats 6917, 6918 and 6924) is considered less reliable. See the discussion for details.

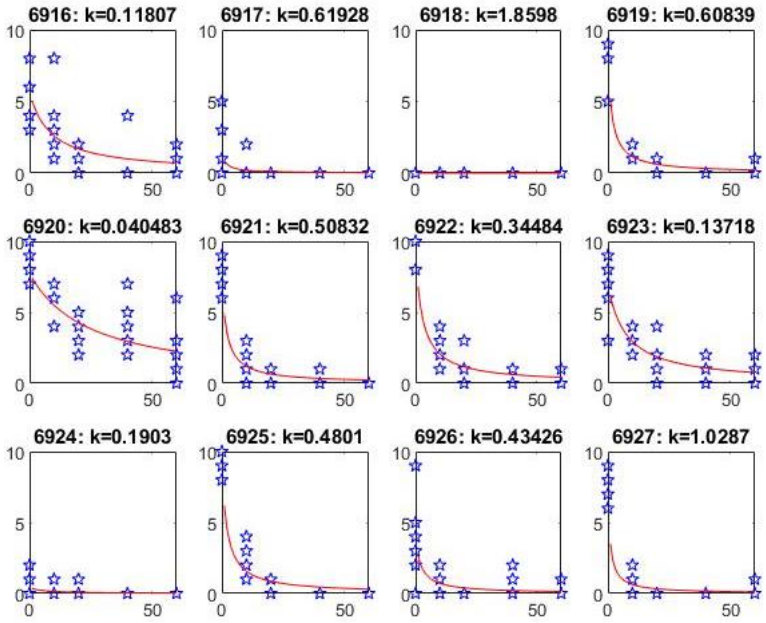

Figure 8: Delay discounting data (number of delay lever selections as a function of delay) from the last seven days of testing. The blue stars indicate the number of selections of the large/delayed reward at each delay, which is fit to the hyperbolic function for each rat, as seen illustrated by the red line.

For the two schedule preference task experiments, the FR value required to achieve equal choice between the FR and RR levers was always much lower than the corresponding RR value. In Experiment 1, we found that the FR value where the rat chose between both levers equally existed somewhere between FR-7 and FR-22 (mean = 13.8699, standard error of the mean (SEM) = 1.06234), referred to as the "equivalence point". The majority of rats would rather work on an RR-50 schedule than an FR-20 schedule, as seen in Figure 9A ($t_{(11)}$ = -34.010, $p < 0.001$). In Experiment 2, even with the RR lever becoming less enticing by adjusting its ratio in the opposite direction, the equivalent FR values were still much lower, with final FR values between FR-14 and FR-37 (and corresponding RR values between RR-86 and RR-63; mean = 19.8785, SEM =

1.90698). In this case, most rats would still prefer to work on an RR-80 schedule than an FR-20 schedule ($t_{(11)} = -15.796$, $p < 0.001$).

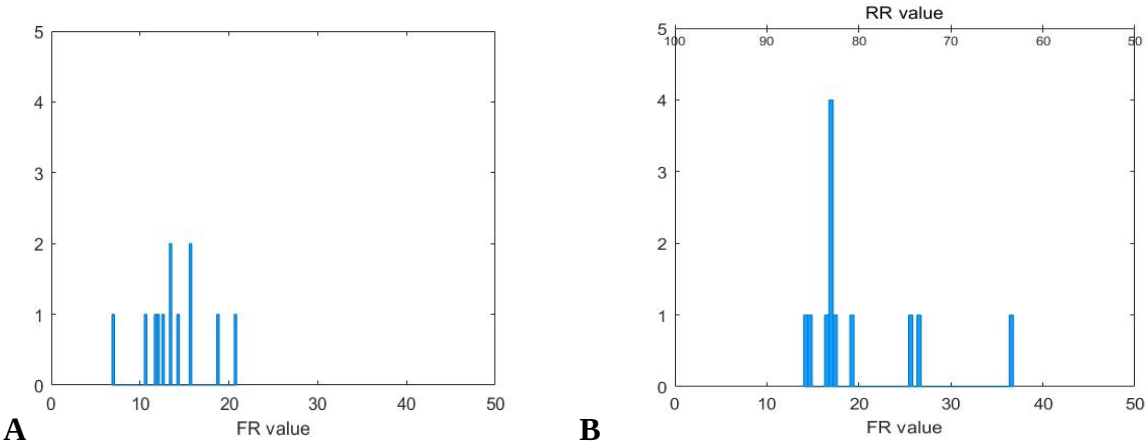

Figure 9: Histograms of FR values when rats choose equally between the FR and RR levers for each experiment. In A, the FR value is the equivalence point when compared to an RR-50 schedule. In B, the FR value is the equivalence point when compared to an RR schedule where the RR value is equal to 100 minus the FR value.

When we compared the data from the delay discounting task and the schedule preference task in Experiment 2, we found that there was no significant effect of k value on the equivalent FR value, but there was a trend in the direction we hypothesized: as the k value increased, the equivalent FR value decreased ($r = -0.163$, $n = 12$, $p = 0.613$). In Figure 10, we omitted the rats that chose the large reward less than 50% of the time at the 0 second delay and it did not change the significance ($r = -0.151$, $n = 9$, $p = 0.698$).

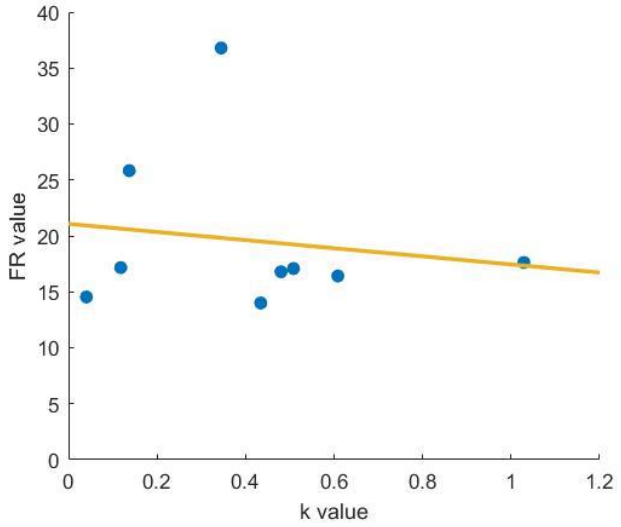

Figure 10: Each animals' equivalent FR value as a function of their k value, and the corresponding line of best fit.

Discussion

In Experiment 1, we found that the rats showed a strong preference for the RR schedule over the FR schedule. However, a weakness of this task is that the RR value remains fixed while the FR value varied. Hence, a savvy rat might purposefully choose the RR lever until the FR value was very low and then alternate, resulting in the maximum payout. To address this possible cheating method, we designed Experiment 2, in which the RR value adjusted in the opposite direction of the FR value. The results of Experiment 2 confirmed that our findings were in fact due to schedule preference and not because of a reward optimization strategy. We found no statistical relationship between choice impulsivity and preference for the RR schedule, but we did show a trend where increased choice impulsivity was negatively correlated with the equivalent FR value in the schedule preference task, suggesting that impulsive rats have a tendency to value the RR schedule more.

The rats showed a marked preference for the RR schedule, and given what other researchers have reported about delay discounting in rats compared to humans, their performance in the schedule preference task was expected. Madden et al. (2007) had previously predicted the degree to which a problem gambler would prefer to work on an RR schedule compared to an FR schedule, based on reported k values for those with and without gambling addiction, and suggested that problem gamblers may find that the subjective value of the gambling option is increased by as much as 376%. It is estimated that the k value, which represents the degree of delay discounting, of a person without addiction is typically under 0.03/month, while someone suffering from addiction may have a k value between 0.03/month and 0.43/month (Alessi & Petry, 2003). Meanwhile, rats tend to discount delayed rewards even heavier, resulting in an estimated k value between 0.2/second and 0.7/second, depending on the strain (Wilhelm & Mitchell, 2009). It is worth noting that human studies typically assess delay discounting using a questionnaire which covers delays of much greater magnitude than those seen in rat testing paradigms. Presumably, a human who shows a steeper discounting over hypothetical delays of months would also be more impatient over the shorter delays seen in gambling. The data collected in these experiments supports Madden's theory that impulsivity plays a role in the preference of gambling-like schedules of reinforcement, as seen in the trend between higher k values and lower equivalent FR values, however, further studies are needed to confirm that this trend is significant.

In Experiment 2, we chose to adjust the RR value in the opposite direction that we adjusted the FR value. The fear was that a savvy animal might "hack" the task by pushing the FR value down and then alternating between the two levers, which would result in the least amount of required lever presses. The downside to this modification is that we could no longer use frozen pseudo-random schedules. Hence, each animal's experience on the RR schedule was uniquely

determined by their choices on the task. In the past, we have used frozen pseudo-random schedules because we can ensure that all animals have the same experience and that the intervals between rewards are not so long that the rats lose interest. To compensate for this problem, we added the constraint that no trial could exceed 200 required lever presses. This constraint created another issue: by capping the work requirement at 200, we changed the perceived RR value when the ratio was greater than 30. The large work requirement between some rewards is necessary in order for there to be many rewards requiring little work, so capping the work requirement meant that the subjective experience of an animal might be that it is working on a lower ratio than it is intended to be working on. We addressed this problem by adding a second constraint: when the RR value was above or equal to 30, the RR value had to be transformed so that the cap did not artificially "water down" the intended ratio requirement. The reason that this only applied to ratio requirements greater than 30 was because, below this value, there are so few intervals above 200 that the schedule is the same with or without the adjustment. The important point is that, even with this adjustment, which resulted in radically high random ratios (e.g., RR-80), all rats still showed a strong preference for an RR schedule given equal work requirements.

The results from the delay discounting task should be interpreted with caution due to a technical issue encountered when conducting the study. When given the choice between a small reward or a large reward, both delivered immediately, the rational choice would be to take the large reward every time. Troublingly, there were three animals that chose the large reward with no delay less than 50% of the time. We ruled out issues with the programming of the schedule, and with the equipment itself, but found that the pellets were still not dispensing reliably. While the pellets smelled good and seemed to be enjoyed by the rats, it became clear that the pellets had begun to lose their structural integrity because they were slightly past their expiry date. This

resulted in them crumbling and clogging up the pellet dispensers, ultimately leading to the unreliable dispensing of the reward. If a rat ended up receiving less pellets than intended after being subjected to a delay, it would be even less reason for the rat to choose that lever again, which is why we think we saw rats that were biased towards the immediate lever at all levels of delay.

Most of the literature that looks at preference for RR over FR schedules does so by comparing response rates when working on these schedules, not through choice paradigms: it has been demonstrated time after time that animals show more vigorous responding on RR schedules (Ferster & Skinner, 1957); rats have faster response rates on RR schedules than FR schedules (Mazur, 1983); and that response rates on RR schedules, which are similar to response rates on FR schedules at low ratio requirements, increase at higher ratio requirements in pigeons (Madden, Dake, Mauel, & Rowe, 2005). Although human performance on RR schedules has been shown to be influenced by a variety of factors, humans also tend to have faster response rates on RR than fixed schedules (Reed, 2020). A few studies have used concurrent choice paradigms to study the preference for adjusting delays in rats (Mazur, 2007) and pigeons (Mazur, 1986). Consistent with the present results, these studies showed that both rats and pigeons prefer varying delays over a single fixed delay. Another study looked at how pramipexole, a dopamine agonist, influenced preference for variable versus fixed reward schedules, and found that pramipexole significantly increased the selection of the VR schedule (Johnson et al., 2011). There has only been one study that has looked at the relationship between impulsivity and preference for probabilistic rewards; while the rats preferred the variable option, there was no significant relationship between preference and delay discounting, although there was significance in a smaller follow-up study (Madden, Francisco, Brewer, & Stein, 2011). This work is important because there is lots of

evidence suggesting that impulsivity and preference for random rewards are related, but the only study that tested similar ideas lacked the sample size required to achieve significance.

Another beneficial aspect of this design is that studying the relationship between impulsivity and gambling in rodents has the potential for longitudinal studies that are not possible in humans; how do you study the factors that may precede problem gambling in people who are not problem gamblers yet? A series of longitudinal studies that looked at impulsivity in adolescence and successive gambling activity in early adulthood found that higher impulsivity (as determined by the Eysenck impulsivity scale) was predictive of subsequent gambling involvement (Vitaro et al., 1997, 1999; Vitaro et al., 1998) but the threshold for gambling involvement was low (Madden et al., 2007). Further, human longitudinal studies, such as these, are extremely resource-intense. Utilizing animal models could help answer the question about what comes first: impulsivity or addiction.

Conclusion

In line with decades of research about reinforcement schedules, we found that Long Evans rats strongly preferred working on a random-ratio schedule (compared to a fixed-ratio schedule). We presented a novel task which measures the degree to which these animals prefer these schedules and showed that this preference may be related to delay discounting. This research has the potential to explain the connection between impulsivity and the appeal of gambling-like schedules of reinforcement; this task provides a new method for studying this relationship and will further our ability to understand and treat gambling addiction.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION

The introduction to this thesis outlined how overlapping dysfunctions in the neurotransmitter systems and brain circuitry between gambling addiction, depression, and impulsivity may underly the cooccurrence of these disorders. We have made the case for the use of animal models to learn about aspects of the gambling experience and have provided two sets of experiments that did just that. Gambling addiction in its entirety may be too complex to model in rodents, but when broken down into its components, there is much potential to learn about the neural correlates underlying this disorder, the causal links between traits and addiction (i.e., does impulsivity precede addiction, or does addiction induce impulsivity), and how we might better help those struggling.

In Chapter 2, we have shown that compulsive reward-seeking behaviour presents itself more in depressed animals. However, this is not a result of the schedule of reinforcement, which weakens the appeal of this paradigm as a model of the emotionally vulnerable pathway to gambling addiction. One particular strength of our approach is that it allows for an assessment of the compulsive aspects of addiction, but our follow-on tests, such as the progressive aversion and reinstatement tasks, could be paired with other models of depression as well.

`There are other ways of modeling depression in rats, like administering repeated corticosterone injections or by subjecting animals to chronic mild stress, that may result in more vulnerability to the random-ratio schedule, which should be investigated. Adverse childhood experiences increase the likelihood of subsequently developing addiction; animal studies looking at the effects of maternal separation and quality of maternal care have suggested that the dopaminergic and glucocorticoid systems are changed by early life stress – neural systems that are also implicated in depression and gambling addiction (Kim et al., 2017). There is evidence that

neuroinflammation, which appears to be related to both depression (Dean & Keshavan, 2017) and addiction (Harricharan, Abboussi, & Daniels, 2017), may alter dopamine transmission.

While the WKY rats did not seem as motivated to obtain reward, they did have trouble inhibiting the behaviour. People with depression are motivated to enter dark flow states (via gambling or other repetitive behaviours) to experience relief from their negative ruminations (Dixon et al., 2018). One explanation for why the WKY rats showed compulsive reward-seeking behaviour, albeit on both schedules of reinforcement, is that they were also experiencing a dark flow-like state. It is unclear whether rats can enter a flow state and whether they might use such a state to mitigate negative feelings, but it is interesting that our rats behave as though they are engrossed in the task, as seen in their decreased sensitivity to increased punishment and to the external cues predicting reward unavailability. While it is unlikely that rats need to escape negative ruminations, our finding suggests that there might be other components of depression that are shared between animals and humans that are relieved by repetitive behaviours like these.

In Chapter 3, we presented a novel task for studying the preference of random-ratio schedules, shown how rats dramatically prefer the gambling-like schedule, and provided preliminary evidence for how this might have a greater influence on those animals who are more impulsive. One of the strengths of this task is its translational value: delay discounting tasks are already implemented using both rats and humans, and it is easy to imagine a human version of the schedule preference task. One potential issue is how the animals might perceive the task. If the FR value is constantly adjusting, is it truly a fixed-ratio schedule? In a new version of the task, we have slowed the adjustment rate down by half, which should make the FR values appear more predictable. Ultimately, our results support the idea that rats are perceiving the FR schedule as different from the RR schedule, or the equivalent FR values would not be so low.

Although we are fairly certain that the issues we experienced in the delay discounting task were precipitated by expired pellets, there is no denying the role that shipping stress must have also had on the animals. The animals in the second impulsivity experiment spent extra time in transit, on account of the COVID-19 pandemic interfering with shipping times and routes. One major consideration going forward, especially when studying traits like anxiety and impulsivity, is how we are going to deal with shipping stress. These "experimentally-naïve" animals have inadvertently been put through a chronic mild stress paradigm before they even arrive, which fundamentally changes their neurobiology, as discussed in previous chapters. Breeding inhouse or sourcing animals from a closer institution, while less convenient and definitely more expensive, might be something to consider going forward with experiments like these.

The literature reviewed supports the idea that depression and impulsivity play a role in problem gambling, and our results are consistent with these previous findings. Our motivation for running these experiments was to better understand the relationship between these particular factors and reward-seeking behaviour. The relationship between depression and problem gambling may not be synergistic, because our "depressed" rats were equally likely to develop compulsion on an FR or RR schedule. Our results do suggest, however, that depression results in a vulnerability to getting caught up in repetitive activities. People with depression (Dean & Keshavan, 2017) and WKY rats (Millard, Weston-Green, & Newell, 2020) both have HPA axis dysfunction; perhaps this hyper-stressed state is what results in the need for some kind of escape, and repetitive activities like gambling (or lever pressing) just happen to be a perfect means to achieve that feeling. Based on the very weak trend that we observed between steeper delay discounting curves and lower equivalence point in the schedule preference task, it may be the case that impulsivity also fuels maladaptive gambling behaviour, by putting an emphasis on the schedules of reinforcement that

offer more immediate rewards, though further study is clearly needed. Preliminary evidence from our research, and others like Madden (2011), suggests that we might need a large sample size in order to have the statistical power to make this possible. If lesioning areas of the rat brain that increase delay discounting, like the nucleus accumbens (Cardinal et al., 2001) or basolateral amygdala (Winstanley et al., 2004), also led to increased preference for the RR lever within our schedule preference task, it would provide compelling evidence of this relationship, while also potentially requiring less animals to do so.

When considering dopamine's role in the reward circuit, and its influence on motivation, it is not surprising that dopamine dysfunction may have an underlying role in many forms of addiction. A reduction in striatal D2/D3 receptors has been implicated in long term substance abuse (Volkow, Fowler, Wang, & Swanson, 2004), but it might not be a feature of behavioural addiction, as there was no difference between problem gamblers and healthy controls in striatal D2/D3 receptor availability (Clark, Boileau, & Zack, 2019; Clark et al., 2012). Paradoxically, D3 receptor availability in the substantia nigra is positively correlated with gambling severity (Boileau et al., 2014). There is evidence that altered dopamine function may be a risk factor for addiction (Leyton, 2017); and gambling, substances, shopping, pornography and gaming all have the power to modulate the reward system and increase dopamine concentrations (Harricharan et al., 2017). Amphetamine use can lead to sensitization of the dopamine system, resulting in increased dopamine release in response to conditioned stimuli; gambling-like schedules can sensitize rats to amphetamine too, suggesting that they operate via similar neural mechanisms (Zack, Featherstone, Mathewson, & Fletcher, 2014) – possibly through the D1 receptor (Zack et al., 2017).

Although gambling addiction does not necessarily mean reduced striatal D2/D3 receptor availability, it does appear that impulsivity and striatal D2/D3 receptor availability are negatively

correlated, and impulsivity is a risk factor for developing both substance and gambling addiction (Clark et al., 2019; Clark et al., 2012). The downregulation of D2/D3 receptors leads to decreased activity in areas like the orbitofrontal cortex and the anterior cingulate gyrus, which is associated with decreased inhibition and increased impulsivity (Volkow et al., 2004). In delay discounting tasks, dopamine signalling in the nucleus accumbens core appears to track magnitude and delay (Saddoris et al., 2015). Rats that are more impulsive have lower cue-related dopamine release in the nucleus accumbens; these concentrations also varied less across delays (Moschak & Carelli, 2017). Depleting dopamine in the nucleus accumbens leads to preference for smaller rewards that require less effort (Salamone, Cousins, & Bucher, 1994). Together, this is compelling evidence that striatal D2/D3 receptors may be the mechanism by which impulsivity influences addiction.

Motivational anhedonia, as seen in depression, is mediated by dopamine projections from the ventral tegmental area to the nucleus accumbens and from the substantia nigra to the dorsal striatum (Der-Avakian & Markou, 2012). There are reports of dopamine agonists improving mood in patients with Parkinson's disease, who experience degradation of the dopaminergic projections in the substantia nigra (Leentjens, 2011). Dopamine agonists have also demonstrated some success against treatment-resistant depression – but there is also evidence of dopamine antagonists improving depression symptoms (Dailly, Chenu, Renard, & Bourin, 2004). Its possible that being in a hypodopaminergic state is what drives people with depression to seek out activities that facilitate dopamine release (like drugs and gambling), but considering the contradictory findings, e.g., the effects of dopamine antagonists, the dopamine theory is considered by many to be outdated. Researchers are now examining other mechanisms, like stress and inflammation, for their roles in the how depression and addiction may interact with each other on a neural level (Harricharan et al., 2017; Kim et al., 2017).

This research is important, because creating animal models with translational value, like the rGT and rDDT, is going to be the way that we learn more about the neurobiology of disordered gambling, while also providing a framework for testing behavioural manipulations and pharmacological treatments for gambling disorder. Behavioural addictions also provide a way to study addiction without the confound of the toxic effects of substances of abuse. Future directions should consider manipulations that target the brain networks and neurotransmitter systems underlying impulsivity, schedule preference and gambling-like behaviour. As mentioned above, these tasks could be very valuable for their longitudinal application and have great translational potential. The experiments outlined here provide more evidence that factors implicated in the pathways model, such as depression and impulsivity, influence reward-seeking behaviour in not only humans, but rats too. How these factors are related on a neural level can be hypothesized but is still unclear, and desperately requires future study in order to appreciate and treat gambling addiction in its entirety.

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