Opposing Effects of Acute Stress in a Rodent Model of Gambling Behaviour

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
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Abstract

Impulsive decision-making is characteristic of both pathological gambling and depression. It remains unclear why some individuals are prone to make risky decisions and develop gambling behaviours. Stress is known to increase the risk for addiction, depression, and disrupt optimal decision-making. The goal of this study is to explore the effects of stress-induced depressive behaviours on gambling-like decision-making in rodents. We hypothesized that stress will increase impulsive decision-making in rats. Rodents were exposed to acute stress using a stress paradigm known to induce depressive-like behaviours and the animal’s decision-making was assessed using a rodent model of gambling-like behaviour. As expected, we found that inescapable footshocks increased impulsive decision-making. Surprisingly, we found that escapable footshocks improved decision-making. Furthermore, high levels of baseline impulsivity in addition to inescapable footshocks increased impulsive decision-making. Our findings suggest that the observed effects of both types of shocks on decision-making could be modulated by baseline levels of impulsivity.
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List of Abbreviations

ACC – anterior cingulate cortex
BIS – Barratt Impulsiveness Scale
DA – dopamine
dLPFC – dorsolateral prefrontal cortex
DS – dorsal striatum
EIS – Eysenck Impulsivity Scale
GDT – game of dice task
HCs – Healthy Controls
HPA – hypothalamic-pituitary-adrenal
ICD – Impulse Control Disorder
IGT – Iowa Gambling Task
LH – Learned Helplessness
MD – Major Depression
NIAAA – National Institute on Alcohol Abuse and Alcoholism
NORC – National Opinion Research Center
PFC – prefrontal cortex
PG - Pathological Gambling
PTSD – post traumatic stress disorder
rGT – rat gambling task
rGT-FC – rGT forced-choice session
SOGS – South Oaks Gambling Screen Scale
SUD – Substance Use Disorder
TCI – Temperament and Character Inventory
VMPFC – ventromedial prefrontal cortex
VS – ventral striatum
Chapter 1: Introduction

1.1 Background and rationale

Problems associated with gambling were first proposed as a health concern in 1972 by Dr. Robert Custer (Korn, 2000). He coined the term ‘compulsive gambling’ to collectively define any problems related to excessive gambling behaviour. In 1980, the American Psychiatric Association officially introduced the term ‘Pathological Gambling’ (PG) in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III: American Psychiatric Association, 1980). In the DSM-IV-TR (2000), PG is defined as the “failure to resist impulses to engage in persistent and maladaptive gambling behaviour despite its disruptive personal, familial, and occupational repercussions” (APA, 2000).

Since its first inclusion in the DSM, PG had been categorized as an Impulse Control Disorder (ICD). In the recently published DSM-5, PG has been reclassified as a behavioural (i.e. substance-less) addiction on the basis that it shares many symptoms and diagnostic criteria with those of Substance Use Disorders (SUDs). For instance, PG and SUD share common elements such as the loss of control and the compulsive engagement in the substance/behaviour despite its harmful consequences, along with symptoms of withdrawal and tolerance (APA, 2000). Similar to individuals with SUDs, pathological gamblers experience cravings for the ‘feel’ of gambling, develop tolerance in which they need to gamble with increasing amounts of money to reach the desired level of excitement, and exhibit withdrawal-like symptoms or become anxious and restless after initial abstinence from gambling (APA, 2000).

Although the majority of adults have gambled at some time in their lives (NGISC, 1999; Wiebe et al., 2006), only a minority develop significant gambling problems. Recent estimates
show that the past-year prevalence of PG in Canada ranges between 1.8-2.4%, whereas in Ontario it is estimated that 2.4% of the population meet diagnostic criteria for PG (Williams, Volberg and Stevens, 2012). Amongst homeless and prison populations, prevalence estimates of PG are significantly higher – 25% (Matheson et al. 2014) and 33% (Turner et al., 2013), respectively.

Genetic association studies of PG estimate that genetic factors account for 35-54% of the liability for developing any symptom of PG (Eisen et al., 1998; Slutske et al., 2010) and the proportion of variance accounted for by genetic factors is directly correlated with the number of DSM criterion met for the disorder. PG and Major Depression (MD) frequently co-occur, with comorbidity rates of up to 49% being reported (Petry, Stinson, & Grant, 2005). Consistent with these findings, Kennedy et al. (2010) shows that 12.5% of patients from a large mood disorder clinic also met diagnostic criteria for PG – a rate over five times higher than current prevalence estimates of PG in the general population. Also, the mood disorder was the primary onset condition for 71% of those who were diagnosed with PG in this study. This high co-occurrence of MD and PG is thought to be influenced by overlapping genetic factors. A large twin study shows that shared genetic factors explain 34% of the PG and MD comorbidity (Potenza et al., 2005). The hypothesis that mood disorder may act as a vulnerability factor in the development of PG is further supported by a large population study in the United States which shows that a history of MD confers an odds ratio of 6.6 for the development of PG (Kessler et al., 2008). Nevertheless, little is known about the mechanisms underlying the PG and MD comorbidity.

The objective of this thesis is to investigate the role of impulsivity and stress in the association between PG and MD through the combined use of animal models of gambling behaviour and depression. In the following sections, we will discuss the evidence from human and animal
studies regarding the role of impulsivity and stress in the development of PG which guided the
development of our hypothesis.

1.2 Pathological gambling subtypes

Clinical research and practice has long documented heterogeneity in the manifestation and
course of PG. This heterogeneity renders the effectiveness of treatments variable across patients.
In their seminal 2002 manuscript (Blaszczynski & Nower, 2006), Blaszczynski and Nower
integrated developmental, neurobiological, cognitive, and personality data to derive a theoretical
model of PG (“the pathways model”), which attempts to explain why some individuals are more
vulnerable to develop gambling problems than others. This theoretical biopsychosocial
framework outlines factors underlying three etiological, but not mutually exclusive, routes to PG,
which have been empirically supported (Milosevic & Ledgerwood, 2010). These subtypes are
derived based on causes of and motivations for gambling as well as concurrent psychopathology
and personality features and include: the behaviorally conditioned, the emotionally vulnerable,
and the “antisocial-impulsivist” (sic) gambler.

The ‘behaviorally conditioned' subtype is characterized by the presence of cognitive
distortions and processes (e.g., poor decision-making skills) without premorbid features of
psychopathology or personality disorder. These gamblers present the least degree of severity and
have the most positive prognostic outcomes in response to cognitive treatments. The ‘antisocial
impulsivist’ subtype is characterized by features of impulsivity, such as impulsive decision-
making, disinhibition, risk-taking, as well as adventure seeking with the constant desire to
increase arousal. This subgroup of gamblers is also likely to present behavioral dysregulation,
including substance use and attention deficit hyperactivity disorder features due to their inability
to control impulses. Of particular interest to the present study is the ‘emotionally vulnerable’ PG subtype. This subtype of PG is thought to use gambling as a means to regulate emotion. These gamblers exhibit depression and anxiety, as well as maladaptive coping strategies.

Together, the aforementioned studies suggest that depression and impulsivity play a role in the development of PG, at least for a subtype of pathological gamblers.

1.3 Neurobiology of pathological gambling

Accumulating evidence on the neurochemical basis of PG has consistently implicated the potential role of dopamine (DA) in the etiology of this disorder and polymorphisms in the DA receptor genes have been linked to PG (Ibanez et al., 2003; da Silva Lobo et al., 2007). Particularly, the mesolimbic dopaminergic pathway is thought to be one of the main neural substrates underlying the development and maintenance of addictive disorders including PG.

1.3.1 The mesolimbic reward system

The mesolimbic reward system is comprised of dopamine neuron projections from the ventral tegmental area (VTA) into the striatum including, the nucleus accumbens (NAcc) of the ventral striatum (VS), caudate nucleus, putamen, along with the amygdala, and the dorsal and ventral prefrontal cortex (PFC) (Baxter & Murray, 2002; Breiter & Rosen, 1999). Dopamine is the main neurotransmitter of the brain reward circuitry and is released from the VTA and transmitted to the NAcc and other areas in the striatum, which contain high concentrations of DA receptors. Under normal conditions, DA stimulations of the brain reward system, in particular the D2 receptors, mediate incentive motivation, increased arousal, and reward-related processes including decision-making (reviewed in Blum et al., 2012).
Multiple lines of neuroimaging evidence have demonstrated increased activity in the mesolimbic DA system in response to rewarding events (Knutson & Cooper, 2005; Yacubian et al., 2006). Clinical findings report increased activity in the NAcc, ventral caudate, putamen, amygdala, VTA of the reward system in response to rewarding stimuli such as anticipated monetary rewards and punishments (Schott et al., 2008; Knutson et al., 2001), substances of abuse such as cocaine (Breiter et al., 1997; Koob, 1992), alcohol, and opiates (Koob, 1992). Comparative findings in rhesus monkeys have demonstrated that primary rewards, such as food, elicit signalling in the VTA dopamine neurons that project into the NAcc (Schultz et al., 1993). Furthermore, different structures within the reward system process reward-related information in different manners. For instance, the VS is involved in the anticipation of future rewards, while the ventromedial prefrontal cortex (vmPFC) is implicated in reward-seeking and goal-directed behaviours (Gläscher et al., 2009). Moreover, the amygdala is involved with learning associations between stimuli and an expected reward or punishment, which plays an important role in decision making (Hampton et al., 2007).

1.3.2 Reward system dysfunctions in pathological gambling

A considerable amount of research demonstrates that individuals with SUDs and PG exhibit dysfunction within the reward circuitry (Volkow et al., 2002; Reuter et al., 2005). Several psychological theories of addiction have been proposed to explain the possible underlying mechanisms of addictive behaviours. Of particular interest to this study, two models have been briefly outlined. These models have different neurobiological substrates but they both involve the mesocortico-limbic dopaminergic reward system.

The reward deficiency hypothesis (Blum et al., 1996; Comings & Blum, 2000) proposes that a hypo-dopaminergic state predisposes individuals to engage in addictive behaviours in an attempt
to temporarily compensate for this deficiency. In this deficient state, naturally-rewarding stimuli are not strong enough to sufficiently activate the brain’s reward circuitry. Consequently, individuals may seek more exciting experiences, such as drug-taking, to compensate for this deficiency. In line with this notion, pre-clinical research report that decreased mesolimbic DA function is correlated with vulnerability to drug-seeking behaviours across several species (reviewed in Nestler, 1993). Clinical evidence also reports that individuals with SUDs exhibit reduced striatal DA receptor density and therefore deficient DA transmission. Insufficient DA activity is thought to increase engagement in reward-seeking behaviours such as, drug consumption and gambling, to compensate for the existing reward deficient state (Comings & Blum, 2000; Blum et al., 2000). Unfortunately, DA transmission is further reduced with repeated drug use, decreasing sensitivity to the pleasurable experience of drugs. Despite these findings, this theory cannot fully address causality; specifically, whether the deficient dopaminergic state precedes development of addictive behaviours or whether it is the result of chronic substance use.

The incentive-sensitization theory of addiction (Robinson & Berridge, 1993; 2001) proposes that repeated drug use sensitizes the dopaminergic response and changes the neural networks that typically regulate the incentive salience to stimuli, a psychological process that is involved in motivated behaviour (Robinson & Berridge, 1993; 2008). In this process, repeated coupling of drugs use with their associated environmental stimuli acquires increased salience and thereby promote drug-seeking behaviour (Robinson & Berridge, 1993; 2001).

By analogy to substance addiction, it has been suggested that PG may be related to deficiency and dysregulation in the reward system. Despite the consistent findings in substance addiction, the role of the dopaminergic reward pathway in PG is not yet clearly understood since the current literature reports inconsistent findings. A relatively small number of neuroimaging
investigations, however, have shown that pathological gamblers exhibit abnormal neural responses to monetary cues alike those found in individuals with substance (Pearlson et al., 2007) and alcohol dependence (Wrse et al., 2007; Beck et al., 2009). Diminished activation of the VS and vmPFC has been reported in PG in response to gambling-related cues such as monetary rewards (Reuter et al., 2005), monetary losses (de Ruiter et al., 2009), and reward/punishment anticipation of gains and losses (Balodis et al., 2012). Specifically, a fMRI investigation by Reuter et al. (2005) reported that, compared to controls, pathological gamblers exhibit lower ventral striatal and vmPFC activity in response to monetary gains during a gambling card-guessing game. Compared to healthy controls, PG individuals show lower vmPFC activation when receiving money, suggesting reduced sensitivity to rewarding stimuli. The degree of deficits in vmPFC activation is negatively correlated with severity of gambling symptoms; hence, hypoactivation of the vmPFC is associated with increased gambling severity (Reuter et al., 2005). Complementing this study, de Ruiter et al. (2009) utilized a probabilistic reversal-learning task to investigate the effects of reward and punishment on subsequent behaviour. During the reversal phase of this task, previously acquired stimulus-reward associations are reversed and new reward/punishment contingencies are introduced; thus, subjects need to adjust their strategy accordingly. The inability to learn the new reward/punishment contingencies reflects response perseveration (de Ruiter et al., 2009). It was reported that, unlike controls, pathological gamblers exhibit severe response perseveration which is accompanied by reduced activity in the ventrolateral prefrontal cortex (VLPFC) in response to monetary gains and losses. Additionally, planning abilities and executive functions are intact in PG individuals as indicated by optimal activity in the dorsal frontostriatal circuitry during the Tower of London task (de Ruiter et al., 2009); suggesting that the abnormal reward processing is not likely due to impaired executive functioning.
Contrary to these findings, a recent PET imaging investigation reported that PG individuals do not exhibit lower striatal dopamine D2/D3 receptor availability compared to controls (Clark et al., 2012); which is in contrast to drug addiction, where reductions in D2/D3 receptor availability has been consistently reported (Volkow et al., 1996; Wang et al., 1997; Fehr et al., 2008). Complementing this study, Boileau et al. (2013) reported no significant difference in D2/D3 receptor levels in the striatum or substantia nigra between healthy controls and PG individuals. Additionally, it was reported that D3 receptors correlates with gambling severity and impulsiveness (Boileau et al., 2013). In a follow-up investigation, Boileau et al. (2014) investigated DA release in response to oral amphetamine administration in PG individuals. The authors reported that PG individuals exhibit greater DA release in the dorsal striatum than healthy controls (Boileau et al., 2014). Moreover, the DA response to amphetamine is positively predicted by D3 receptor levels and correlates with gambling severity (Boileau et al., 2014). This hyperdopaminergic state in response to amphetamine reported in PG individuals is in agreement with the incentive-sensitization theory of addiction (Robinson & Berridge, 1993; Boileau et al., 2014). In line with this notion, pharmacological studies investigating the DA mechanism in PG report increased ventral striatal DA transmission in response to reward delivery during various gambling tasks (Schott et al., 2008; Steeves et al., 2009). Increased DA release in response to reward-related stimuli is also associated with PG symptom severity (Joutsa et al., 2012) and increased levels of excitement in pathological gamblers (Linnet et al., 2011). These findings are in contrast with substance addiction literature, where reduced DA transmission in response to stimulant challenge is consistently reported. Therefore, PG may not be associated with a blunted DA transmission as in SUDs but instead it may be associated with an increased DA response, which could be related to the engagement in rewarding acts (Boileau et al., 2014).
Taken together, the current literature on reward system dysfunctions in PG reports inconsistent findings; while there is some evidence of blunted neural response to rewarding and punishing stimuli in PG individuals (de Ruiter et al., 2009; Reuter et al., 2005); recent neuroimaging studies report a hyperdopaminergic response upon stimulant challenge. These findings suggest that, despite the phenomenological commonalities between PG and SUDs, the underlying neurobiological substrates between substance and behavioural addictions may differ.

1.4 Impulsivity

Impulsivity is another factor considered to play a role in the development and maintenance of pathological gambling (Alvarez-Moya et al., 2010). Impulsivity is broadly defined as “actions that are expressed on acute impulse to satisfy an urge,” which are often characterized by poor judgement and risk-taking in the pursuit of rewards, and ultimately result in impaired decision-making and goal-directed planning (Evenden, 1999). Comprehensive reviews of the literature on impulsivity and addictions suggest that impulsivity affects various stages of addiction (Kreek et al., 2005). For instance, variations in impulsive traits such as, elevated risk-taking and novelty-seeking may maintain the initial act of drug use and facilitate the progression to chronic drug use (Kreek et al., 2005). By analogy to substance use, it is possible that elevated impulsivity may cause a subset of individuals to seek out rewarding activities such as casual gambling, may contribute to the persistence of the behaviour and facilitate the progression to compulsive gambling. In line with this notion, there is a growing body of research consistently indicating that pathological gamblers exhibit elevated impulsive traits such as, impaired decision-making (Brand et al., 2005), loss of control (Sylvain et al., 1997), preference for immediate rewards at
the expense of net loss (Cavedini et al., 2002), novelty- (Martinotti et al., 2006), and sensation-seeking (Blaszczynski et al., 1986).

Longitudinal investigations also show that impulsivity during childhood and adolescence significantly predicts the development of PG later in life (Slutske, Caspi, Moffitt, & Poulton, 2005; Vitaro, Arseneault, & Tremblay, 1999). Furthermore, elevated self-reported impulsivity in PG is associated with increased gambling severity (Alessi & Petry, 2003; Blaszczynski et al., 1997; Steel & Blaszczynski, 1998) and increased depression and psychological distress (Blaszczynski et al., 1997; Steel & Blaszczynski, 1998).

Complementing these reports, principal component analyses examining variables associated with PG such as, impulsivity, depression, anxiety, and erroneous beliefs, report that within the group of PG subjects, the impulsivity component significantly associates with the most severe level of gambling pathology (Turner et al., 2008).

Impulsive behaviours are broadly classified into two distinct forms: *impulsive action* and *impulsive choice* or decision-making. ‘Impulsive action’ reflects the inability to inhibit or delay an inappropriate response, whereas ‘impulsive choice’ refers to disadvantageous or risky decision-making that favours big immediate and less advantageous rewards over delayed but more advantageous ones (Ainslie, 1975).

**1.4.1 Impulsive choice/decision-making**

Making decisions in general is a key function of everyday life. Decision-making under uncertainty, in particular, involves choosing between options associated with expected but unknown/uncertain probabilities of rewards/losses (Bechara, 2004). A variety of cognitive and emotional processes along with environmental factors such as stress influence decision making. This complex mental process involves risk-taking, flexibility in behaviour, reflecting on the
positive or negative outcomes of a certain choice, continuous evaluation of immediate against delayed gratifications and punishments, integrating information about the expected magnitudes and probabilities of various options, and ultimately deducing the most optimal strategy that will likely result in the maximal benefit (Krawczyk, 2002; Goudriaan et al., 2006; Bechara et al., 2004). Dysfunction in processes associated with decision-making is a characteristic of several psychiatric disorders, including fronto-temporal dementia (Rahman et al., 1999), depression (Must et al., 2006), and PG (Cavedini et al., 2002).

Neuroimaging studies conducted on healthy subjects and patients with focal brain lesions have provided important information regarding the neural circuitry that is involved in decision-making. Circuits including the limbic loop encompassing the ventromedial prefrontal cortex (vmPFC), the amygdala, the ventral striatum (VS), the insular cortex, as well as the cognitive loop including the dorsolateral prefrontal cortex (dLPFC), in particular the anterior cingulate cortex (ACC), and the dorsal striatum (DS) have shown to be involved in reward-motivated decision-making under uncertainty (Tanaka et al., 2004; Ernst & Paulus, 2005; Clark & Manes, 2004; Krain et al., 2006). These two systems exert different levels of control over decision-making. The limbic loop is generally involved with responding to potential rewards/losses as well as emotional control, whereas the cognitive system, which receives input from the limbic loop, is implicated in long-term planning (Bechara, 2005; Brand et al., 2006). The vmPFC, for instance, is involved with integrating information from the limbic loop pertaining to the evaluation of positive/negative outcomes, all of which play an integrated role in deducing the best long-term decision-making strategy (Krawczyk, 2002; Bechara et al., 1999; Brand et al., 2007).

Lesions to such regions, in both humans and rats, have shown to impair stable decision-making and result in increased preference for impulsive/disadvantageous options associated with
long-term losses (Bechara et al., 2004; Paine et al., 2013). For instance, individuals with vmPFC lesions have demonstrated insensitivity to future outcomes and impulsive decision-making, characterized by a persistence preference for small immediate rewards over larger delayed ones regardless of the long-term losses (Bechara et al., 2004).

1.4.2 Impulsive decision-making in PG

PG is characterized by the lack of self-regulation and repeated participation in impulsive or risky decision-making marked by an increased preference for high-rewarding options (van Holst et al., 2010). Human gambling typically involves cost-benefit comparisons between options with differing amounts of risk and reward. The magnitude and probability of receiving risks/rewards are uncertain and are only determined to a certain extent through trial and error (Brand et al., 2007). A commonly used laboratory task of human decision-making that fulfils this requirement is the Iowa Gambling Task (IGT) (Bechara, Damasio, & Anderson, 1994). In this task, participants are required to repeatedly choose a card from one of four decks that differ in terms of the frequency and magnitude of monetary rewards and punishments. Two decks are associated with smaller rewards but also low rates of penalties (‘conservative/non-risky decks’), which results in a higher net monetary gain in the long-run. In contrast, the other two decks deliver larger rewards but also higher rates of large penalties (‘risky decks’), which results in a net loss over time. Subject’s choice distribution across trials reflects their tendency toward risk-taking and their ability to evaluate future consequences. Therefore, the IGT provides a reliable measure of impulsive decision-making or the preference for risky (disadvantageous) over safe (advantageous) options (Bechara et al., 1994).

There is a growing body of literature indicating impulsive decision-making under gambling-like schedules of reward and punishment contingencies in pathological gamblers as assessed by
the IGT (Cavedini et al., 2002; Goudriaan et al., 2008; Kertzman et al., 2011). These studies generally report that PG individuals demonstrate impaired IGT performance marked by a preference for the risky/disadvantageous decks that are associated with higher gains but also higher losses (Cavedini et al., 2002; Goudriaan et al., 2008; Kertzman et al., 2011). Unlike healthy controls, PG individuals typically fail to shift choice preference to the advantageous decks as they receive feedback from their previous choices, indicating a decreased ability to optimally evaluate the long-term outcomes of their decisions (Cavedini et al., 2002). PG individuals also display ‘chasing losses’ in which they are less likely to change decks after experiencing a series of losses in hopes of recuperating their losses (Goudriaan, Oosterlaan, de Beurs, & van den Brink, 2005). PGs are often more impulsive and faster at responding than HCs, which further contributes to their impaired performance. Moreover, PG individuals exhibit diminished skin conductance and heart rate changes in the anticipation of disadvantageous decks (Goudriaan et al., 2006), which may indicate a possible psychophysiological deficit in response to losses/punishments, which in turn likely exacerbates their preference for high reward/high losses decks.

Similar results have been obtained from studies utilizing other types of decision-making tasks such as delay discounting tasks. In a typical delay discounting task, subjects are required to make a choice between two rewards that vary in size and time of receipt (Myerson & Green, 1995). The smaller-immediate reward is considered as the ‘impulsive choice’, while the larger-delayed reward is considered the ‘self-controlled choice’ (Ainslie, 1974). Increased preference for the smaller-immediate rewards reflects higher reward-based impulsivity and lower self-control (Critchfield & Kollins, 2001). High rates of delay discounting have been reported in pathological gambling in laboratory settings (Petry, 2001; Petry & Casarella, 1999) as well as in natural gambling settings (e.g. off-track betting facility for horse racing) (Dixon et al., 2003).
Interestingly, substance abusers with a history of gambling problems exhibit higher rates of delay discounting than both SUD individuals without gambling problems and healthy controls (Petry & Casarella, 1999); which indicates additive effects of comorbid substance abuse and PG on decision-making. Furthermore, gambling severity positively correlates with delay discounting rates (Alessi and Petry, 2003). Not surprisingly, positive associations between self-reported impulsivity scores and delay discounting rates have also been reported in pathological gamblers (Alessi and Petry, 2003).

Though not fully known, it has been suggested that this pattern of impulsive decision-making demonstrated by PG individuals may stem from their hypersensitivity to immediate rewards. One study investigating the specific cognitive parameters involved with decision-making on the IGT reported that PG individuals are more intrigued by options resulting in immediate gains. It has been demonstrated that in PG individuals, signals triggered by immediate rewards carry more weight than those triggered by losses (Brevers et al., 2014). So, PG individuals may flag options that result in higher immediate gains as more significant compared to those that provide lower rewards, which can drive a risky decision-making preference (Brevers et al., 2014).

In summary, there is evidence that pathological gamblers present impulsive decision-making compared to non-pathological gamblers marked by difficulties evaluating the long-term consequences of their decisions and a failure to modify or improve behaviour in response to previous feedback.

1.4.3 Impulsive decision-making in MD

An increasing body of IGT studies in MD individuals demonstrate that they exhibit impaired risk/reward decision-making alike those found in PG (Must et al., 2006; Cella et al., 2010). These studies report that, similarly to PG, some MD individuals show a preference for the risky
decks that provide immediate but smaller rewards on the IGT (Must et al., 2006; Cella et al., 2010). Despite the resulting net loss, MD individuals fail to shift choice selection and to develop a long-term beneficial strategy. Other investigations using a variant version of the IGT similarly report impaired performance in MD subjects (Cella et al., 2010). This version of IGT includes a ‘contingency shifting’ phase in addition to the standard IGT procedure, where previously-learned contingencies shift values. Similar to PG individuals, MD subjects have difficulties shifting previously-learned selection patterns towards advantageous decks as a result of previous feedback (Cella et al., 2010).

Considering the difficulty to change choice strategy even after receiving repeated punishments, it has been suggested that MD individuals may have an impaired ability to optimally integrate reward-related feedback history in guiding future decisions (Must et al., 2013). The impaired performance on the IGT has been suggested to stem from the decreased sensitivity to rewards or punishments associated with the clinical symptoms of MD (Must et al., 2006).

Investigations using various monetary paradigms indicate that individuals with high levels of depressive symptoms (Henriques et al., 1994) and those diagnosed with MD (Henriques & Davidson, 2000) show reduced responsiveness and motivation to obtain rewards. Pizzagalli et al. (2008) employed a probabilistic reward task which assesses the subjects’ ability to modulate performance based on previous reward reinforcements (i.e. reward responsiveness) and found that MD individuals show reduced reward responsiveness marked by the inability to modulate performance based on previous feedback. Interestingly, MD individuals are responsive to a single immediate reward; however, they show impairments in integrating reinforcement history over trials in order to develop the optimal strategy toward the more frequently-rewarded stimulus
(Pizzagalli et al., 2008). This impairment is correlated with self-reported anhedonia (Pizzagalli et al., 2008).

In accord with these findings, neuroimaging studies have demonstrated structural abnormalities and activity in the brain reward system of depressed individuals in response to reward-related stimuli (Forbes et al., 2006; Dichter et al., 2012). Specifically, reduced cortical volumes in caudate, putamen, and amygdala have been reported in depressed individuals (Beyer and Krishnan, 2002). Moreover, diminished neural activity in response to monetary rewarding stimuli in the striatum region such as the bilateral putamen and nucleus accumbens has been reported (Pizzagalli et al., 2009; Steele et al., 2007). Elliott et al. (1996) demonstrated that, while performing the Tower of London task, depressed individuals exhibit reduced activity in the medial caudate and ventromedial OFC in response to both positive and negative feedback. Using a monetary incentive delay task, Pizzagalli et al. (2009) examined the neural responses of depressed individuals to positive and negative feedback and reported that depressed individuals show reduced response to monetary gains in the left nucleus accumbens (i.e. reduced activation). Similarly, Steele et al. (2007) reported that depressed individuals show impaired response to positive (i.e. monetary gain) and negative (i.e. monetary loss) feedback information during a gambling task which is accompanied by diminished VS activity. The impaired performance to positive and negative feedbacks in this study is correlated with self-reported anhedonia (Steele et al., 2007). In line with these studies, a recent investigation reported that remitted depressed individuals show abnormal neural responses to both reward anticipation and outcomes (Dichter et al., 2012) along with reduced activity in the left inferior and superior frontal gyri in response to both loss anticipation and outcomes (Schiller et al., 2013). Interestingly, the reduced neural activity in response to loss was correlated with rumination, which is the tendency to perseverate on negative emotions (Schiller et al., 2013).
In addition to IGT impairments reported in depressed individuals, findings from the aforementioned studies indicate that individuals affected by MD are less responsive to reward and loss-related information and are unable to optimally modulate behaviour according to prior reinforcements to guide subsequent decision-making. These behavioural phenotypes correspond to abnormal structural and neural activity in the brain reward regions. Therefore, there is evidence that some MD individuals do exhibit impulsive decision-making under uncertainty that resemble impairments found in PG individuals.

1.5 Modelling impulsive and gambling-like decision-making in rodents

There is a growing interest in the use of animal models of impulsive decision-making in exploring the neural underpinnings of gambling-like behaviours. It is challenging to design a model of a highly comorbid and heterogeneous disorder, such as PG, and to capture its entire symptom profile. Instead, successful models of PG focus on its core phenotypes such as impulsive decision-making. Decision-making in rodents has been modelled in many different ways including: delay-discounting tasks (smaller-sooner over larger-delayed reward) (Monterosso and Ainslie, 1999), probabilistic delivery tasks (larger-riskier over smaller-safer reward) (Adriani & Laviola, 2006), and the rodent analogues of the IGT (Rivalan et al. 2009; Zeeb et al. 2009; Van Den Bos et al., 2006). These tasks essentially involve the cost-benefit analysis of several options that differ in immediate or long-term gains and losses. Just as in humans, gambling-like behaviour in rodents is modelled by the choice between a low-probability but high-payoff option over a high-probability but low-payoff alternative. In animals benefits/rewards are typically presented as palatable food, while costs/punishments are presented as delays before reward, shocks, or unpalatable foods.
1.5.1 Delay-discounting task

In the delay-discounting task rodents are required to choose between a small reward delivered immediately (smaller-sooner) or a larger reward delivered after a time delay (larger-delayed). This task essentially assesses the subject’s self-control and ability to make advantageous choices by delaying immediate rewards (Monterosso and Ainslie, 1999). Typically, subjects are expected to shift preference from the larger-delayed to the smaller-sooner rewards overtime (Adriani et al., 2006). The optimal strategy is to choose the larger-delay reward, which is advantageous in the long-run. Preference for the smaller-sooner rewards over the more beneficial delayed ones indicates impulsive decision-making.

1.5.2 Probabilistic delivery task

The probabilistic delivery task is another approach to assess impulsive decision-making by varying the probability and uncertainty of reward delivery (Adriani & Laviola, 2006). In this task, animals have to choose between a smaller but certain reward (i.e. safe) and a larger but uncertain (i.e. probabilistic) one. The larger-uncertain reward is delivered occasionally and randomly such that the subject faces a loss and the accumulation of losses overtime decreases the long-term gain. Rats initially learn to favour the larger reward over the smaller one; however, this option eventually becomes risky since the probability of its delivery decreases progressively. Therefore, to maximize net gain, rats have to shift the previously-developed preference for the larger reward to the small-certain one. A majority of rats typically shift preference towards the small-certain reward, which is a safer option as the probability of receiving the larger reward declines (Zoratto et al., 2014). However, a subset of them show gambling-like proneness or impulsive decision-making, which is marked by the sustained preference for the larger-uncertain
rewards that ultimately results in a lower net gain (Zoratto et al., 2014). Although the delay
discounting and probabilistic delivery tasks both assess impulsive decision-making, the latter
resembles features of gambling more closely since the probability of obtaining reward is
uncertain. In the delay discounting task, the delivery of rewards is certain even after a delay;
however, in the probabilistic delivery task, waiting for a larger reward is uncertain and
probabilistic.

1.5.3 rat Gambling Tasks (rGT)

The aforementioned rodent models are useful in assessing certain aspects of decision-making;
however, they do not fully encapsulate certain principles underlying decision-making in the
context of human gambling. For instance, they do not fully incorporate elements of risk and
uncertainty which is a core aspect of human gambling. As pointed out, human gambling involves
the constant cost-benefit comparisons between options with different amounts of reward and
loss. The risk associated with each option is unknown and is determined through trial and error to
a certain extent. Furthermore, these tasks fail to accurately reproduce the sensation of loss in the
context of gambling. The risk of losing the resources staked, such as money, when the wager is
unsuccessful is a crucial aspect of human gambling (Zeeb et al., 2009).

Recently, new rodent models of gambling-like decision-making have been developed based
on the human IGT that incorporates the uncertainty of reward and punishment outcomes in
human gambling. Van Den Bos et al. (2006) designed the earliest rat Gambling Task (rGT) in
which rodents choose amongst four arms with varying contingencies of rewards (sugar food
pellets) and punishments (quinine/bitter pellets). The ‘advantageous’ arms deliver small
immediate rewards but yield a high net gain; in contrast, the ‘disadvantageous’ arms deliver
larger immediate rewards but yield a lower net gain. In this task, it is argued that punishment is signaled by the delivery of bitter food pellets; thus, rodents are likely choosing between rewards based on their appetitive quality (Zeeb et al., 2009). Incorporating loss in this manner does not accurately represent the sensation of loss in human gambling since there is no risk of negative payoffs and animals cannot finish a session at a disadvantage compared to the start (Zeeb et al., 2009).

The rat Gambling Task (rGT) utilized in this study is thought to present the most significant parallels to human gambling-like behaviour and decision-making under uncertainty (Zeeb et al., 2009). In this task, rats choose among four different options, analogous to the four decks of cards, each associated with varying probability and magnitude of food rewards and punishing timeout periods. Through trial and error, they learn the schedules of contingencies associated with each option; two of which are linked with larger food rewards but higher probability of punishments (i.e. the ‘high-risk’ or ‘impulsive options) and the other two options are linked with smaller rewards but also smaller probability of punishments (i.e. the ‘low-risk’ or ‘optimal’ options). The optimal strategy in the rGT mirrors that of the IGT, which is to favour options the low-risk options resulting in a higher net food gain overall per session. Consistent selection of the ‘high-risk’ options, on the other hand, is ultimately disadvantageous and results in a smaller net food gain. In addition to impulsive decision-making, the rGT concurrently measures impulsive action (motor impulsivity), which is another aspect of impulsive behaviour.

The rGT incorporates the nature of risk and uncertainty in human gambling and requires animals to evaluate the long-term costs and benefits for each option. Moreover, the sensation of loss involved in human gambling is better reproduced in this task. The rGT incorporates loss through the use of punishing timeout periods, during which food cannot be earned. Time is
therefore the resource at stake since longer penalty timeouts reduce the chance to earn more food during a given test session. Rats have to essentially balance the desire for larger food rewards with the risk of longer penalty periods. The rat’s rGT choice distribution reflects its level of impulsive decision-making (i.e. its ability to identify the optimal choice and to avoid high-risk alternatives) and increased preference for the risky options reflects impulsive decision-making.

The rGT is suitable for pharmacological manipulations aimed to assess the underlying mechanism of decision-making. Current research elucidating the underlying neurobiology of impulsive decision-making using the rGT report that amphetamine impairs decision-making whereas dopamine D2 receptor antagonist eticlopride increases the selection of the optimal rGT option (Zeeb et al., 2009).

The rGT is an interesting model of cross-species decision making. Similar to findings from clinical studies using the IGT (Bechara, 2004), rGT studies report that the majority of rodents are able to deduce the optimal strategy and essentially learn to maximize rewards by avoiding the high-risk options (Zeeb et al., 2009). In a similar rat gambling task (also based on the IGT), it has been shown that a subset of rats persistently prefer the high-risk options (Rivalan et al., 2009), alike pathological gamblers (Cavedini et al., 2002), which is indicative of impulsive decision-making. Thus, the assumption in the rat gambling tasks derived from the IGT is that rats that show preference for large immediate rewards exhibit a behavioural profile similar to PG in which they are more sensitive to larger immediate rewards and less sensitive to punishments (Rivalan et al., 2009).
1.6 Modelling depression in animals: the role of stress models

Stress has long been recognized as a contributing factor in the etiology of various psychopathologies including, addiction (Lloyd & Turner, 2003), depression (Brown, Bifulco, & Harris, 1987), and as a factor impacting cognitive processes, such as decision-making and memory (Mendl, 1999).

1.6.1 Stress and vulnerability to addictions and depression

Exposure to stressful events increases vulnerability to addictive behaviours (Harlow et al., 1986) and negative emotional states (Seligman & Maier, 1967). One explanation for the significant link between stress and addiction is the self-medication hypothesis; where some individuals who have experienced stressful life events may begin abusing substances in an attempt to alleviate negative emotional distress and cope with the tension associated with life stressors (Khantzian, 1997). Ample clinical research demonstrates a positive association between adverse life events and chronic distress with increased addiction vulnerability. Physical abuse, for instance, is associated with early onset of substance use and with an increased risk of the subsequent development of addiction (Liebschutz et al., 2002). Substance abuse rate in combat veterans suffering from PTSD is also significantly higher compared to veterans without PTSD (McFall et al., 1992). Alcohol consumption is also positively associated with stress levels. Individuals who use alcohol as a coping mechanism to deal with stress are more likely to develop alcohol addiction compared to social drinkers (Cronkite & Moos, 1984; Cooper et al. 1992).

Substantial animal research similarly demonstrates that exposure to both acute or chronic stress, especially early in life, enhances drug-seeking behaviours. Stressors such as social isolation, footshocks, and restrain are reported to enhance self-administration of alcohol (Derr &
Lindblad, 1980), cocaine (Kosten & Miserendino, 2012), and opiate across several species (Le et al., 2006). For instance, adult rhesus monkeys raised by peers (stressed condition) consume significantly higher amounts of alcohol as opposed to those reared by their biological mothers (Higley et al., 1991). Interestingly, mother-reared monkeys confronted with stressors later in life, such as social separation, increase their alcohol consumption to the same level of peer-reared monkeys, while peer-reared monkeys do not change their alcohol consumption. Prenatal stress, in the form of mental or physical stress, in rodents has been linked to increased substance abuse during adulthood (Deminière et al., 1992). The offspring of rodents exposed to prenatal restraint stress, for instance, exhibit higher levels of amphetamine self-injection later in life (Deminière et al., 1992). Exposure of adult rodents to repeated tail-pinching (Piazza et al., 1989) and uncontrollable footshocks (Goeders & Guerin, 1994) has also been reported to facilitate drug-seeking and self-administration behaviours and enhance sensitivity to amphetamine and cocaine (Goeders, 2002). Exposure to stress also promotes drug relapse even after a long period of extinction or withdrawal. Exposure to inescapable footshocks induces the reinstatement of heroine self-administration in rodents even after prolonged periods of extinction (Shaham & Stewart, 1995).

Another possible mechanism linking stress and addiction is related to sensitization. Preclinical literature on stress and addiction suggests that exposure to stress changes the brain reward circuitry and enhances sensitivity to the reinforcing effects of drugs all which contribute to the maintenance of addictions (Koob & Le Moal, 1997). Pre-clinical literature indicates that stress and drug exposure increase mesolimbic dopamine activity (Abercrombie et al., 1989). For instance, daily injection of cortisol, a stress hormone, increases sensitivity to cocaine, similar to the effects caused by environmental stressors (Goeders, 2002).
In summary, the existing clinical and pre-clinical literature on stress and addiction suggests that stress modulates drug-seeking behaviours and the transition to addiction and restores previously-extinguished addictive behaviours. Exposure to stress may increase sensitivity to the rewarding effects of drugs and individuals who are particularly sensitive to stress may engage in substance abuse to suppress stressed-induced symptoms of anxiety or depression (Piazza & Le Moal, 1998).

Exposure to stress and emotional trauma has also been implicated in the etiology of depression (Kaplan & Klinetob, 2000). Personal experiences such as loss of job, friends, or events over which one has little control, may lead individuals to perceive that one is powerless and attempts to cope with additional stressors seem useless. The resulting negative cognitive state interferes with learning new coping skills to deal with future challenges, produces persistent feelings of sadness, and increases susceptibility to other illnesses that in turn worsen the existing conditions (Overmier & LoLordo, 1998).

Pre-clinical studies have demonstrated that exposure to stress result in behavioural changes that resemble symptoms of depression (Seligman & Maier, 1967; Weiss & Simson, 1986; Krishnan et al., 2007). Early research by Overmier & Seligman (1967) shows that animals with previous exposure to inescapable shocks have difficulties in learning to avoid subsequent shocks that are escapable. Animals with no prior inescapable shock exposure, on the other hand, learn to normally avoid the escapable shocks (Overmier & Seligman, 1967). Animals with prior exposure to inescapable shocks passively accept subsequent escapable shocks and make no effort to escape them when given the opportunity because they expect that no instrumental response will terminate the shocks (Overmier & Seligman, 1967). Previous exposure to inescapable shocks interferes with acquiring the normal escape response and decreases the motivation to respond in an escapable situation. In addition to poor escape learning, exposure to inescapable shocks
results in other behavioural deficits such as, increased anxiety and decreased social interaction (Short & Maier, 1993), reduced social dominance (Rapaport & Maier, 1978), reduced food and water intake (Weiss, 1968), and other symptoms that closely resemble those observed in depressed patients such as ulcer formation and lethargy (Willner, 1984). These behavioural changes are collectively termed ‘learned helplessness (LH)’ (Maier & Seligman, 1976) and ‘behavioural depression’ (Weiss et al., 1980). The learned-helplessness effect has been widely utilized as an animal model of depression (Seligman, 1975) and anxiety-related disorders such as PTSD (Weiss et al., 1980; Maier, 2001). Consistent with this notion, several classes of antidepressant administrations have been reported to reverse some of these stress-induced behavioural deficits (Sherman & Petty, 1980; Martin et al., 1987).

1.6.2 Stress responsivity

Considering the prevailing evidence, it is important to note that stress is not sufficient to cause depression or addiction per se. Most individuals do not become depressed following stressful events (Nestler et al., 2002). Krishnan et al. (2007), for instance, demonstrated that a subset of inbred mice exposed to aggressive and larger animals over a period of 10 days, display depressive-like behaviours such as, decreased social interactions and sucrose preferences (i.e. susceptible mice), while the rest of them do not exhibit such deficits (i.e. resilient mice). It is not yet fully understood why some mice are resistant to stress-induced behavioural deficits. Recently, it has been suggested that abnormal stress reactivity or responsivity may increase the vulnerability to the development of addictions or depression in both humans and animals (Schepis et al., 2011; reviewed in Clarke & Schumann, 2009; Burke et al., 2005).

Stress typically activates the hypothalamic-pituitary-adrenal (HPA) axis and induces the release of stress hormones such as cortisol (Sapolsky, 1996). Disruptions in the HPA axis impair
the ability to respond adaptively to stress. A growing body of clinical literature shows that HPA axis activation or suppression influences the course of addiction (Kreek et al., 2005). Former heroin addicts, for instance, exhibit a hypo-responsive HPA system, whereas former heroin addicts with ongoing cocaine dependence show a hyper-responsive HPA system (Schluger et al., 2001). Similarly, compared to non-depressed controls, depressed individuals exhibit elevated levels of cortisol, a reliable indicator of the HPA stress axis, following exposure to a stressor (Gold et al., 1986; Young et al., 1994). Moreover, corticotropin-releasing factor (CRF), which is involved in the HPA stress response, is elevated in depressed individuals and contributes to HPA dysregulation and behavioural symptoms of depression (Swaab et al., 2005). Complementing these clinical findings, pre-clinical research in rodents indicate that following exposure to social stress, induced by the resident-intruder model of defeat, a subgroup of a healthy population of rats exhibit neurochemical and behavioural phenotypes that resemble depression (i.e. shorter defeat latency or submissive/passive defeat posture) along with elevated CRF secretion. Interestingly, the remaining subgroup of rats is resistant to such behavioural depression (i.e. longer defeat latency or upright/proactive posture) and exhibit decreased efficacy of CRF (Wood et al., 2010). Since the two subgroups were from the same strain of rats, it is likely that genetic variability underlies the vulnerability or resilience to such stress-induced depressive-like behaviours (Wood et al., 2010).

Therefore, pre-clinical and clinical evidence report that individual differences in the response to stress are associated with different neurochemical and behavioural consequences. From this perspective, depressed individuals may be more sensitive or reactive to the impact of stressful events than non-depressed individuals. So, it is likely that some individuals are biologically more reactive to stress and may be more vulnerable to develop depression or addiction in response to stressful events.
1.6.3 Stress and decision-making

Stress has been reported to disrupt cognitive processes such as decision-making and working memory (Mendl, 1999). The effect of stress on decision-making is mediated by corticosteroid hormones released in response to stress. As pointed out, stress responses are partly mediated by the HPA axis, which control the release of neurochemicals like cortisol into the blood (Sapolsky, 1996). The glucocorticoid receptors are ubiquitously expressed in the prefrontal cortex (PFC) and the limbic regions (Diorio et al., 199; Ahima & Harlan, 1990; Reul & Kloet, 1985; reviewed in McEwen et al., 1986); so, it is likely that stress can exert modulating influences on emotional as well as cognitive processes. Although it is unclear exactly how stress impacts subsequent decision-making, brain regions implicated in decision-making, such as: the PFC, OFC, and amygdala (Pruessner et al., 2010), are known to overlap with those that are involved with stress reaction and are therefore sensitive to stress-induced changes.

As reviewed by Starcke and Brand (2012), studies addressing the neural correlates of stress reveal mixed findings. Some clinical studies report decreased activity in regions of the orbitofrontal cortex (Pruessner et al., 2008) with stress exposure; while some report increase activation in the dlPFC and the VS (Pruessner et al., 2004). These mixed results likely reflect individual differences that may explain why some individuals respond differently to stress than others (Kudielka & Wüst, 2009).

The majority of the clinical investigations of stress and decision-making report more risk taking under acute stress (reviewed in Starcke & Brand, 2012). For instance, using a gambling task known as the Game of Dice Task (GDT; Brand et al., 2005), Starcke et al. (2008) investigated the effects of anticipatory stress on subsequent decision-making. Participants chose among different options of dice that were associated with varying probabilities of gains and
losses. Options associated with high gains are disadvantageous because they are also associated with higher probability of losses. In this study, stress was induced using the Trier Social Stress Test (TSST) by instructing participants to deliver a public speech, which typically increases the activation of the HPA axis (Kudielka et al., 2004). According to the authors, compared to controls, subjects who are stressed due to anticipating a public speech delivery, make more risky and disadvantageous decisions in hopes of potentially higher rewards (Starcke et al., 2008). Interestingly, cortisol levels and risky decision-making were reported to positively correlate. Other clinical studies utilizing the IGT to assess decision-making report similar impairments. For example, stressed individuals are slower at learning the IGT task contingencies and take longer to shift choice strategy towards advantageous decision-making (Preston et al., 2007). In another study, Zhang et al. (2011) investigated the effects of stress on IGT performance in individuals recovering from heroin dependence. According to the authors, before exposure to acute stress, the recovering addicts exhibit IGT performance similar to that of healthy controls. Following stress exposure, however, these addicts demonstrate impaired decision-making and perform significantly worse than controls who were exposed to the same stress (Zhang et al., 2011). The authors suggested that stress worsened an already existing impairment in decision-making.

In line with these findings, the secretion of cortisol in response to acute stress is thought to promote risky behaviour. High cortisol responders are more sensitive to immediate rewards and demonstrate impaired IGT performance than low responders (Van den Bos et al., 2009). It has been suggested that acute abnormal elevation of cortisol levels, in response to stress, is associated with euphoria reward-like properties and sensation-seeking and thus increases risk-taking. Considering the prevailing clinical evidence, stress can indeed promote risky behaviour, influence reward and punishment sensitivity, and ultimately alter optimal decision-making.
Pre-clinical research on the direct effects of stress on decision-making has been quite limited and often conflicting. One study demonstrated that exposure to chronic and unpredictable stress in rodents negatively affects risk-assessment (Wang et al., 2014) and increases impulsive choice during adulthood. Moreover, chronic stress during adolescence in rodents is found to induce a long-term negative cognitive bias, which consequently enhances sensitivity to loss and thus impacts the interpretation of ambiguous stimuli (Chaby et al., 2013). Contrary to the effects of chronic stress during early life, pre-clinical research on the effects of acute stress on decision-making has been somewhat conflicting. One study, reports no significant change in decision-making following acute stress (Shafiei et al., 2012), while a recent study reports increased preference for the impulsive choice following corticosterone administration as assessed by a rodent gambling-like task (Koot et al., 2013).

1.7 Aim of the present study

Based on the foregoing review of what is known in the literature, there is evidence to suggest an underlying association between pathological gambling, depression, and elevated levels of impulsivity. Impulsive traits have been reported to mediate the effects of depressive emotional vulnerability on the severity of gambling (Clarke, 2006), and preliminary findings suggest that as levels of depressive symptoms increase, the severity of gambling also increases as a function of impulsivity (Clarke, 2006). Neuroimaging investigations of PG report abnormal sensitivity to reward and punishments along with heighten reward-seeking behaviours (Reuter et al., 2005; Boileau et al., 2013). Similarly, depressed individuals display anhedonic symptoms and a general blunted sensitivity to natural rewarding stimuli (reviewed in Naranjo et al., 2001). The
imbalances in reward sensitivity and reward circuitry along with impulsive traits may underpin impulsive behaviour and decision-making reported in MD and PG.

Moreover, pre-clinical and clinical literature has long established the role of stress in the vulnerability to addiction and depression and its effect on cognitive processes such as decision-making. Exposure to stress in both humans and animals has been associated with increases in drug-seeking and addictive behaviours, depressive-like behavioural deficits such as learned helplessness, and risky decision-making (Goeders, 2002; Weiss, 1968; Preston et al., 2007; Koot et al., 2013). Some critical questions, however, remain unresolved about the underlying role of stress and depressive behaviours in risk-based decision-making.

To the best of our knowledge, no animal study has attempted to clearly demonstrate and investigate the direct effects of stress and depressive-like behaviours on subsequent gambling-like decision-making. This is potentially a rich subject for exploration since human gambling is closely linked to decision-making processes. Understanding the roles of stress and depressive behaviours on decision-making could therefore provide valuable insight into gambling behaviours and other types of addictions.

The present study aimed to investigate the effects of stress-induced depressive behaviours on subsequent impulsive decision-making as assessed using a rodent gambling-like behavioural task. In order to explore this topic, we coupled the Learned Helplessness (LH; Maier & Seligman, 1976) task, known to induce depressive-like behaviours in rodents, with the rat Gambling Task (rGT; Zeeb et al., 2009), that models human gambling and assesses impulsive decision-making under uncertainty. Initially, the rats’ baseline gambling-like decision-making was evaluated using the rGT; they were then exposed to acute stress (inescapable and escapable footshocks) using the LH task; finally, their gambling-like decision-making was reassessed in
order to determine whether stress and depressive-like behaviour had altered their decision-making.

1.7.1 Hypothesis

Based on the existing clinical and pre-clinical literature, we hypothesize that exposure to acute stress will increase impulsive decision-making and gambling-like behaviours. Specifically, rats exposed to stress, using the LH task are expected to present an increase in impulsive decision-making and gambling-like behaviour.

If the hypotheses are confirmed it would suggest that stress interferes with optimal decision-making. Prevention efforts regarding treating gamblers, especially those who gamble to escape depression, could therefore focus on improving coping with stress and decision-making habits, which may ultimately help reduce susceptibility to develop gambling dependence.
Chapter 2: Materials and Methodology

2.1 Subjects

The sample consisted of 45 male Fischer-344 rats (Charles River, Quebec), initially weighing between 250 – 275 grams, at 60 days of age. Males of this strain have shown to gain weight at much lower rates than male rats of the Sprague-Dawley or Wistar strains, which made them ideal for the long-duration experiments of this study (House & Masoro, 2007). Additionally, they are more reliable than other strains for assessing the effects of stress (Dhabhar, McEwen, & Spencer, 1997).

2.2 Instruments

2.2.1 rat Gambling Task (rGT)

Apparatus. The rGT was carried out inside 9 standard five-hole operant conditioning chambers (30.5 x 24 x 21 cm; Med Associates Inc., St. Albans, VT, USA), which were individually housed in ventilated and sound-attenuated cabinets (Med Associates Inc., Vermont). Each chamber contained a series of five response holes (middle response hole was omitted) with a yellow stimulus light placed behind each hole along with horizontal infrared detector beams to detect nose-poke responses. On the wall opposite to these holes, a food tray/dispenser was situated in the midline and was similarly equipped with tray light and infrared beams. The food tray dispensed sucrose pellets (45 mg; Formula P, Bio-Serv, New Jersey, USA) from a connected pellet dispenser placed on the outside of each chamber. The response holes and food tray were positioned 2 cm above a bar floor. Each chamber was also equipped with a fan to provide proper ventilation as well as a white house light, placed in the center of the roof, for proper illumination.
Data was collected automatically via an online MEDPC software (version 1.17; written by Dr. Catharine A. Winstanley), installed on an IBM-compatible computer.

**rGT procedure: habituation & training.** Prior to testing, animals were habituated to the testing chambers for two consecutive days. During each habituation session, sucrose pellets were manually placed inside each of the four response holes and the food tray; this allowed the animals to explore and become familiarized with the chamber. After two days of habituation, rats were trained to make a nose-poke response into an illuminated response hole to earn food reward. The training phase aimed to train the animals to learn the basic operant response and to associate nose-poke into the illuminated response hole with food delivery.

During each training trial, one of the four response holes became illuminated and animals were rewarded when they made a correct nose-poke response into the illuminated hole within a certain length of time. The location of the stimulus light varied pseudorandomly across holes between trials. The training phase consisted of three stages and animals were required to meet the minimum criteria for each stage in order to advance to the next one (Table 1). For instance, during the final stage, animals had to respond within 10-seconds and complete all 100 trials with a minimum of 80% accuracy and less than 20% omissions (a trial was considered omitted if the subject failed to respond within 10-seconds). Animals completed a total of 7 training sessions: 2 days of T1, 2 days of T2, and 3 days of T3.
Table 1. Three stages of the rat Gambling Task (rGT) training phase.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Limited hold (s)</th>
<th>Stimulus duration (s)</th>
<th>Inter-trial interval (s)</th>
<th>Criterion for next stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>30</td>
<td>30</td>
<td>2</td>
<td>≥ 30 correct trials</td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>≥ 30 correct trials</td>
</tr>
<tr>
<td>T3</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>≥ 50 correct trials, ≥ 80% accuracy, ≤ 20% omissions</td>
</tr>
</tbody>
</table>

Rats completed a total of 7 sessions of training each lasting approximately 30 minutes. The aim of the training phase was to train the rats to learn the basic operant response and associate a nose-poke into an illuminated response hole with food delivery. For instance, in Stage T2, one of the four response holes was illuminated for 20-seconds and rats had to make a nose-poke response within 20-seconds in order to correctly complete a trial. A trial was considered ‘Omitted’ if they failed to respond within the Limited hold period. Animals had to meet the criteria for each stage in order to advance to the next. For instance, in order to advance to Stage T3, they had to complete at least 30 correct trials in Stage T2. By the end of Stage T3, animals completed an average of 100 trials with at least 80\% accuracy and less than 20\% omissions.
**rGT Procedure: forced-choice session.** After training, rats completed the rGT forced-choice session (rGT-FC), during which the varying rGT reinforcement schedules associated with each of the four response holes were introduced. During each trial, animals had to make a nose-poke response into a single illuminated response hole. The forced-choice session was similar to the training session; however, in this session after a response was made, the schedule of rewards and punishments associated with the selected response hole was introduced. This session aimed to ensure all animals sampled each of the options and had equal experience with their associating reinforcement contingencies in order to prevent the development of biases toward a particular hole. Rats completed a total of 8 forced-choice sessions, each lasting approximately 30-minutes.

**rGT Procedure: full rGT test session (free choice session).** After the rGT-FC session, animals started testing on the full rGT program or the free-choice rGT. Similarly, in this version, a trial was initiated by a nose-poke into the illuminated food tray. This nose-poke extinguished the tray light stimulus and prompted a 5-second inter-trial interval (ITI). After the ITI, all four response holes (P1, P2, P3, and P4) were illuminated for 10-seconds and subjects were able to freely choose among any of the four illuminated options. This is slightly different from the rGT-FC, where only one response hole was illuminated at a time. After the ITI, a nose-poke response into any of the four illuminated holes turned off all stimulus lights and led to either the delivery of the associated reward or the punishing time-out period depending on the contingency associated with the selected response hole. If the trial was rewarded, the appropriate number of sucrose pellets was immediately dispensed into the food tray. If the trial was to be punished, no food reward was delivered and instead the stimulus light within the chosen hole flashed at a frequency of 0.5 Hz until the end of the punishing time-out period (the lights in the other response holes were extinguished at this time). At the end of the penalty period, the tray light
was re-illuminated so that a new trial could be initiated. A nose-poke into the illuminated food tray always initiated the next trial following either rewards or punishments. As in the rGT-FC, a trial was considered ‘omitted’ if animals failed to respond within 10-seconds, at which point the tray light was re-illuminated and they were able to initiate a new trial. A nose-poke response into any of the holes prior to the end of the ITI was considered a ‘premature response’ and led to the illumination of the house light for a 5-second time-out duration after which the tray light was re-illuminated and a new trial could be initiated. Animals were tested five times a week, each test session lasted 30-minutes, until choice behaviour was stable.

Figure 1 displays the schematic of the free choice rGT task. As illustrated, response hole 1 corresponded to option 4, hole 2 corresponded to option 1, hole 4 to option 3, and hole 5 corresponded to option 2. Option 1 (P1) delivered 1 sugar pellet on 90% of the trials and was associated with a 5-second time-out penalty 10% of the trials; yielding a hypothetical net gain of 295 pellets. Option 2 (P2), the optimal option, delivered a reward of 2 sugar pellets on 80% of the trials and a 10-second time-out period on 20% of the trials; yielding a hypothetical net gain of 411 pellets. Option 3 (P3) was rewarded with 3 pellets on 50% of the trials and a 30-second punishment on 40% of the trials; yielding a hypothetical net gain of 135 pellets. Lastly, option 4 (P4), the high-risk option, delivered 4 sugar pellets on 40% of the trials and a 40-second penalty time-out 60% of the trials; yielding a hypothetical net gain of only 99 pellets. A summary of the schedules of reinforcement is presented in Table 2.2. The position of these holes and their associated schedules of reinforcements remained unaltered for the duration of testing.

Given the 30-minutes allotted to each session, P2 is the optimal choice in terms of reward earned per unit time. The next advantageous option is P1. These two advantageous options are associated with smaller immediate gains (1 and 2 sugar pellets, respectively) but also shorter and less frequent penalty time-outs (P1: 5-seconds; P2: 10-seconds time-out); resulting in a higher
The two disadvantageous options (P3 and 4) are both associated with larger immediate gains (3 and 4 sugar pellets, respectively), but also longer penalty time-outs (P3: 30-seconds; P4: 40-seconds time-out); resulting in a lower net food gain. P4 is the most high-risk and disadvantageous option and leads to a net loss since it was associated with larger punishments and lower probability to complete as many trials and earn as much reward within 30-minutes. Exclusive selection of options associated with either smaller (P1) or larger (P3 or P4) rewards ultimately leads to a lower net food gain due to the associated longer and more frequent punishing time-out periods. Therefore, theoretically, P2 is the optimal option, followed by P1, P3 and then P4.

**rGT behavioural measures.** The rGT choice distribution of each animal was collected using the MEDPC software and was re-coded using SYSTAT for Windows (version: 12.00.08). The percentage of each rGT option selected (P1–4) was calculated according to the following formula: (number of selection of a particular option / number of total selections per session) x 100. This percentage was used to determine the subject’s preferences for each option, which reflected its level of ‘impulsive decision-making.’ The percent of premature responses made during the ITI was also calculated: (number of premature responses made / total number of trials initiated) x 100. This percentage reflected the ability to inhibit an inappropriate or impulsive response (impulsive action). These two behavioural measures have shown to be very stable behavioural traits and suitable for longitudinal assessments. Other responses such as: the total number of perseverative responses made, total number of trials completed, and number of omitted trials per session were also calculated. rGT performance was considered stable when a subject exhibited a stable pattern of choice, trial, and premature responses across the last 3 test sessions.
Figure 1. Schematic of the rat Gambling Task (rGT) procedure.

The task is initiated with a nose-poke response into the illuminated food tray. This response extinguishes the tray light and initiated a 5-second intertrial interval (ITI). After the ITI, the 4 response holes (P1-4) are illuminated and the animal is required to make a nose-poke response into any of these holes within 10-seconds. This response is then rewarded or punished based on the contingency schedule of reinforcement of the selected hole. The p values below each option represent its probability of rewards or punishments. Figure was modified from Zeeb et al., 2009 and Zeeb & Wistanley, 2011.
Table 2. rat Gambling Task (rGT) contingency schedules of reinforcement.

<table>
<thead>
<tr>
<th>rGT Options</th>
<th>Punishing Time-out</th>
<th>Reward Pellets</th>
<th>Hypothetical Maximum Pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probability</td>
<td>Duration</td>
<td>Probability</td>
</tr>
<tr>
<td>P1</td>
<td>0.1</td>
<td>5-sec</td>
<td>0.9</td>
</tr>
<tr>
<td>P2 (optimal)</td>
<td>0.2</td>
<td>10-sec</td>
<td>0.8</td>
</tr>
<tr>
<td>P3</td>
<td>0.5</td>
<td>30-sec</td>
<td>0.5</td>
</tr>
<tr>
<td>P4 (high-risk)</td>
<td>0.6</td>
<td>40-sec</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 2. Each rGT option (P1-4) was associated with varying probability and amount of rewards and punishments. Each option was associated with either a fixed number of food pellets (1 – 4) or a penalty time-out period of certain probability (0.1 – 0.6) and duration (5 – 40 seconds). Therefore, the hypothetical maximum number of food pellets that could be earned during each 30-minute test session can be calculated (Zeeb et al., 2009). Theoretically, P2 was the optimal option since it yielded the highest net food gain; P4, on the other hand, was the high-risk or the most impulsive option since it yielded the lowest net food gain.
2.2.2 Learned Helplessness (LH) Task

The LH task is utilized to model depressive-like behaviours in rodents. This task induces behavioural despair and deficits in coping behaviour, ‘i.e. helplessness’, which is reminiscent of symptoms, observed in clinically depressed individuals (Overmier & Seligman, 1967). This task centers on the theory that prior experience with inescapable traumatic events results in later unexpected behavioural deficits in associative learning and decision-making, behavioural coping, and emotional expression (Overmier & Seligman, 1967). In a typical procedure, rodents are initially exposed to inescapable footshocks and then to escapable footshocks that can be terminated by pressing a lever. Behavioural studies using the LH task have demonstrated that, rats that were not exposed to the initial inescapable footshock session learn to escape footshocks during the escapable session fairly quickly; while animals that were previously exposed to the inescapable footshocks fail to do so. This failure to escape the escapable footshocks is considered a type of stress-induced behavioural depression. Antidepressant interventions have shown to reverse the escape deficit in these helpless rats (Malberg & Duman, 2003).

An important feature of this LH paradigm is that only a proportion of the animals exposed to inescapable footshock show subsequent escape deficits. This is a stable trait that can be selectively bred, as can resistance to helplessness (Shumake & Gonzalez-Lima, 2003). This differential susceptibility to show helplessness after a prior inescapable shock experience is also consistent with the human experience, where not everyone develops depressive-type symptoms after exposure to stressful experiences. Among existing models of depressive-like behaviour, the LH task has proven to have good face validity and good predictive validity (Henn & Vollmayr, 2005), which in turn has made it into an interesting model to investigate the pathophysiology of depression (Vollmayr & Henn, 2001).
Apparatus. The LH behavioural testing took place inside 4 sound-attenuated operant chambers (25 x 30 x 21.5 cm; Med Associates, Georgia, VT, USA), which were individually placed inside ventilated cabinets. Each chamber was composed of clear Plexiglas walls fitted with a floor composed of 37 electrified stainless steel rods 6 mm in diameter which were separated by 1.0 cm. These rods were connected to a software-controlled Coulbourn constant-current shock generator (model: BSR-LVE Sg 904/113-02; Med Associates, St. Albans, VT, USA) that was used to deliver footshocks. On the right side of the chamber, there was a 35 x 35 mm lever mounted 7 cm off the grid floor projecting 6 cm into the cage. A 12W white signal light was placed 5 cm directly above the lever.

LH procedure. Rats were divided into three groups: (1) stress (n = 27), (2) no-stress (n = 10), and (3) control condition (CC) (n = 8) groups. Stress group was initially exposed to an inescapable footshock session followed by an escapable footshock session. The no-stress group only received escapable footshocks, under the same conditions. Control condition group did not undergo the LH procedure and served as controls. The no-stress group intended to verify that learning normally occurs under escapable footshocks for rats that were not previously exposed to inescapable shocks. The shock intensity of the foot shocks was 0.8 mA; which has been reported by past research using similar model (Whitehouse et al. 1983; Tejedor-Real et al., 1995) and was selected based on a shock-intensity response curve.

Inescapable footshock session. On the first day of testing, the stress rats (n = 27) were placed inside the experimental boxes where they received 100 randomized inescapable 0.8 mA footshocks delivered via the electrified steel floor bars. Shocks were delivered as a scrambled pulsed current with durations varying between 1.5 – 60 seconds and the interval between them varied between 1–30 seconds. The onset and offset of the shocks was established by a
probability generator and resulted in a total shock exposure of 25 minutes per animal. Each inescapable shock session lasted approximately 45 minutes. On this day, no-stress and control condition groups remained in their home cages.

**Escapable footshock session.** On the following day, the stress group was placed inside the same operant boxes where they received 15 trials of 0.8 mA escapable footshocks with 24 seconds intertrial intervals in between. The initiation of each shock was associated with the illumination of the white light above the lever where it remained illuminated during the shock period. These shocks were delivered in a pulsating schedule of 35 msec on / 35 msec off. A single lever press terminated the footshock and extinguished the light stimulus. Termination within the first 20 seconds of the trial was recorded as a successful ‘escape response’; otherwise, the trial was automatically terminated at 60 seconds and was classified as a ‘failure to escape’ response. The no-stress rats \( n = 10 \) were similarly exposed to the same escapable footshocks. Each escapable shock session lasted approximately 25 minutes.

**LH behavioural measures.** Latency to escape (in seconds) the escapable footshocks was recorded for each animal over the 15 trials of escapable shock session. Behavioural deficits were measured as the number of failures to escape the shocks. The average escape latency of each escapable shock trial for the stress and non-stress groups was calculated.

**Statistical Analysis.** The stress and no-stress groups’ latency to escape the footshocks during the escapable session was recorded in seconds. Initially, the number of escape responses (i.e. termination of shock within the first 20 seconds) of the two groups was compared using a non-parametric test. Next, the mean escape latencies of the two groups were compared using a non-parametric test; ‘latency to escape’ was entered as a continuous variable, while a ‘group’ categorical variable represented the two groups.
2.3 General experimental procedure

Upon arrival, rats were pair-housed and free fed standard laboratory rat chow for one week, during which the experimenter handled them daily. Water was always available ad libitum in their home cages and their beddings were changed every four days. They were housed in a temperature-controlled colony room on a 12 h light–dark cycle (lights on at 8:00 A.M.; temperature 21°C). Animals were handled daily for an acclimation period of one week and throughout behavioural testing. On day 8, they were separated and housed individually and were food-restricted to 80% of their initial free feeding (i.e. 20-22 grams once per day). Along with standard rat chow, subjects were given 5 grams of sugar pellets (45 mg; Formula P, Bio-Serv, New Jersey, USA) that were used in the rGT in order to become familiar with its taste and texture. Under this schedule rats have been previously shown to remain healthy, increase their body weight steadily, and maintain stable levels of behavioral performance (Fletcher, Chambers, Rizos, & Chintoh, 2009). They were always fed immediately after behavioral experimentations. In order to ensure proper health and growth, animals were weighed on weekly basis. Handling of the animals was always carried out by the same individual. All housing conditions and behavioral testing and procedures were in accordance with the Animal Care Committee at the Centre for Addition and Mental Health and complied with Canadian Council on Animal Care (CCAC) and NIH standards and guidelines prior to the beginning of the study (certificate numbers A06-0451 or A08-0519).

rGT training began one week following the start of food restriction. Animals were tested between 8:00 A.M. and 3:00 P.M. five days per week. On testing days, they were transported to the testing room in their home cage prior to being placed inside the operant chamber. Rats were always placed in the same operant chamber for the duration of behavioural testing. Following 15
days of baseline rGT testing, animals were divided into three groups; where two groups underwent the LH paradigm, while the third group served as controls. After the 2 day stress paradigm, all animals were re-tested on the rGT, for one week, to assess the effects of stress (Figure 2).
Figure 2. Overall experimental design and timeline of the study.

Following one week of acclimatization and one week of food-restriction, animals underwent daily training on the rat Gambling Task (rGT) for one week. Upon meeting the criteria for the training phase, they began the Forced-Choice rGT (rGT-FC) session for 8 days. Afterwards, they completed 15 sessions of the free-choice or full rGT program in order to obtain rGT baseline behaviour. Once animals reached stable responding on the rGT, they underwent stress using the Learned Helplessness (LH) stress protocol. One group of rats completed one session of Inescapable Shock; on the following day, 2 groups completed the Escapable Shock session. Lastly, all animals were re-evaluated on the rGT for one week to obtain rGT post-stress rGT behaviour. The behavioural effects of acute stress were assessed in a between-subjects design.
2.4 Statistical Analyses

The Statistical Package for the Social Sciences (SPSS Inc., version 21, Chicago, IL, USA) was utilized for all data analyses. Kolmogorov-Smirnov test for normality along with histograms confirmed that all variables were normally distributed; therefore, standard parametric tests were employed for all analyses.

rGT choice behaviour was analyzed using 2x2 factorial repeated measures analysis of variance (ANOVA) to compare mean values between and within the three groups, with ‘time (pre-/ post-LH task)’ as the within-subject factor and ‘groups (stress, no-stress, control condition)’ as the between-subject factor. Post-hoc comparisons for significant within-subjects effects were performed using paired sample t-tests and for significant between-subjects effects were performed using ANOVAs. The pre-stress rGT choice behaviour of the HI, MI, and LI groups was analyzed using two-way ANOVA. All tests were two-tailed with the level of statistical significance set at $p < .05$ unless stated otherwise.
Chapter 3: Results

3.1 Pre-stress rGT performance

Animals were tested until baseline behaviour was stable on all rGT parameters across at least three consecutive test sessions. They completed an average of 103.8 ± 6.12 trials per test session. The rGT baseline choice pattern is displayed in Figure 3 and the overall proportions of choice selection are illustrated in Figure 4. Animals displayed varying individual preferences for each option. Pairwise comparisons showed that rats selected options in the following order: P2 > P1 > P4 > P3. They significantly favoured the optimal option (P2) as it represented an average of 71.4% ± .078 of all the choices made per session; followed by P1 (17.9% ± .052), P4 (7.20% ± .012), and finally P3 (3.50% ± .021) (rGT options: $F_{3,56} = 624.2 , p < .001$; P2 vs. P1: $t_{28} = 21.9, p < .001$; P2 vs. P3: $t_{16} = 32.2, p < .001$; P2 vs. P4: $t_{15} = 31.2, p < .001$). This choice pattern was established relatively early and became more pronounced as testing continued and remained generally stable thereafter.
Figure 3. Pre-stress rGT choice distribution.
Baseline rGT choice distribution of the rats (n = 45) for each option (P1-P4) and their premature responses (PRs) are expressed as a mean percent of total choices per session over the course of 15 sessions. During each test trial, every rGT options were illuminated and were available for selection. On average, animals consistently showed a significantly strong preference for P2 and this preference remained constant and became progressively more pronounced with further testing. PRs decreased marginally with more testing, reaching a plateau at approximately 22% of the total choices.
On average, the optimal option (P2) was significantly preferred by the majority of the animals and it represented 71.4% of the total selections made per session (rGT options: \( F_{3,56} = 624.2, p < .001 \); P2 vs. P1, \( t_{28} = 21.9, p < .001 \); P2 vs. P3, \( t_{16} = 32.2, p < .001 \); P2 vs. P4, \( t_{15} = 31.2, p < .001 \)). The least optimal options (P1, P3, and P4) were chosen significantly less frequently than P2 (17.9%, 3.50%, and 7.2%, respectively). As summarized below the bar graphs, since P2 was associated with a small gain but also smaller and less frequent punishments, it earned the highest theoretical number of pellets (411) if selected exclusively.
3.2 Classification of rats

3.2.1 Classification based on baseline impulsivity

After obtaining pre-stress rGT performance, the rats were divided into three groups of (1) low, (2) medium, and (3) high impulsivity, based on the average proportion of P4 choice selection over the 15 rGT sessions. P4 was chosen because it is the most impulsive or high-risk option. A threshold of 7% was chosen; so, rats that chose P4 more than 7% of the time were labeled as high impulsivity (HI); those that chose P4 between 4-7% of the time were labeled medium impulsive (MI); and those that chose P4 less than 4% of the time were labeled low impulsivity (LI) rats. The threshold of 7% was chosen since it was the median of our dataset and there are no comparable studies in the current literature that provide another threshold value. Using this classification system, 11 rats were identified as HI, 6 as MI, and 28 as LI. Figure 5 displays the average choice distribution of each group. As illustrated, at pre-stress rGT performance, HI rats favoured the high-risk option significantly more and the optimal option significantly less than the other two groups.

Of note, due to the 7% threshold, there is a high variability in the percentage of P4 choice within the HI rats (range: 8.00% - 47.8%). In the LI rats, on the other hand, there is a smaller variability (range: 0.00% - 3.20%).

3.2.2 Distribution of baseline rGT choice

After ranking the rats based on their average baseline P4 selection, the pattern of choice selection of every rat throughout the month of testing was further assessed. This was conducted in order to determine where the shift in choice preference occurred for every rat. It is possible that rats, regardless of their baseline impulsivity, increased selection of the option (P4) at the
beginning of testing simply due to their state of hunger, since P4 delivers a higher number of pellets. An example of selection pattern of a HI, MI, and LI rat is displayed in Figure 6. As illustrated, the rats generally displayed a stable pattern of choice selection throughout testing. For instance, the HI rat significantly favoured P4 from the beginning of testing and persisted with this pattern throughout testing. The LI rat, on the other hand, selected either options P1 and P2 at the beginning, however, shifted selection to P2 and maintained this preference for the duration of testing. The fairly stable rGT performance across the sessions suggests that the varying choice preference of the three types of rats was more likely driven by baseline levels of impulsivity rather than their state of hunger.

3.2.3 Learned-helplessness group assignment

As pointed out, prior to the initiation of the LH stress task, rats were randomly assigned into three groups: 27 rats were assigned to the ‘stress’ group; 10 were assigned to the ‘no-stress’ group; and 8 were assigned to the ‘Control Condition (CC)’ group. Group composition (i.e., number of HI, MI, and LI rats) was balanced as much as possible with regards to pre-stress baseline rGT performance to control for the development of possible biases towards a particular option. As illustrated in Figure 7, prior to the induction of acute stress, the choice preferences of the groups were statistically similar to one another (P1: $F_{2,42} = 2.17$, $p = .126$; P2: $F_{2,42} = .864$, $p = .429$; P3: $F_{2,42} = 3.03$, $p = .059$; P4: $F_{2,42} = 8.65$, $p = .001$).
Figure 5. Pre-stress rGT choice distribution of the HI, MI, and LI groups.

One-way ANOVA analyses reported that the high impulsivity (HI) rats ($n = 11$) favoured P4 significantly more than the other two groups. They also selected the optimal option (P2) significantly less than the medium (MI) ($n = 6$) and low impulsivity (LI) rats ($n = 28$). The choice selection of P1 and P3 of the three groups did not differ significantly. Error bars represent the standard error of the mean for each group.
A)

High Impulsivity Rat

B)

Medium Impulsivity Rat
Figure 6. Pre-stress rGT choice distribution of the HI, MI, and LI rats across the 15 sessions.

An example of the pre-stress rGT choice distribution of a (A) HI, (B) MI, and (C) LI rat. As displayed, the baseline rGT choice selection of the rats is fairly stable throughout testing. For instance, the HI rat favoured the high-risk option (P4) from the beginning of testing and maintained this preference. The LI rat, on the other hand, consistently favoured the optimal option (P2) throughout testing.
Figure 7. Pre-stress rat Gambling Task (rGT) choice distribution of the stress, no-stress, and control condition groups.

One-way ANOVA analyses reported that the baseline rGT choice behaviour of the three groups did not significantly differ from one another (P1: $F_{2,42} = 2.17, \ p = .126$; P2: $F_{2,42} = .864, \ p = .429$; P3: $F_{2,42} = 3.03, \ p = .059$; P4: $F_{2,42} = 8.65, \ p = .001^*$). So, prior to the stress paradigm, all three groups had similar rGT choice behaviour and one group did not have a bias towards a particular option. Error bars represent the standard error of the mean for each group.
3.3 Stress vs. no-stress groups’ escape latencies

During the second day of the LH paradigm, both stress and no-stress groups were exposed to escapable footshocks and the number of escape responses was recorded. Termination of shocks within the first 20 seconds of each trial was recorded as an ‘escape response’; otherwise, the trial was classified as a ‘failure to escape.’ On average, stress group had fewer escape responses per session than the no-stress group (1.44 ± .920 vs. 1.90 ± .510, respectively); however, this difference was not significant (independent samples median test, \( p = .787 \)).

Despite the fact that difference in the number of escape responses between groups was not statistically significant, on visual inspection of the results, the steeper slope of the no-stress group indicates that they learned to escape the shocks much faster than the stress group (Figure 8). Also, Figure 8 shows that some individuals were clear outliers in both groups, indicating that the LH procedure was ineffective for some individuals. In order to better clarify the effects the LH procedure, we performed a second analysis excluding the outliers and found that there was a significant difference between groups (independent samples median test, \( p = 0.016 \)). However, by excluding the outliers the size of our no-stress group decreased from 10 to 6 individuals. Therefore, we did not exclude the outliers of the no-stress group.
Figure 8. Mean escape latency trends of stress and no-stress groups across the 15 trials of escapable footshocks.

Latencies to escape for the stress ($n = 27$) and no-stress ($n = 10$) groups across 15 escapable footshock trials. Each point represents the mean (±SEM) escape responses of each group during one session. The latency to escape of both groups gradually decreased with increasing trials. However, the no-stress rats learned to avoid and escape from the footshocks much faster than the stress rats. In other words, the stress group exhibited escape deficits (i.e. learned-helplessness effect). The slope of the linear learning trend of the no-stress rats is steeper than the stress rats, indicating faster learning. Error bars represent the standard error of the mean for each group.
3.4 Effects of acute stress on rGT decision-making

A 2x2 factorial repeated measures ANOVA with between-subject factor: group; and within-subject factor: time (pre- and post-LH task) for each rGT choice was performed. The results revealed a significant interaction between time and group \((F_{2,42} = 7.073, p = .002)\) and a non-significant main effect of group \((F_{2,42} = 1.19, p = .314)\) on P4 choice. There was a significant main effect of time on P1 choice \((F_{1,42} = 9.02, p = .004)\); P2 choice \((F_{1,42} = 19.4, p < .001)\); and P3 choice \((F_{1,42} = 10.4, p = .002)\). There was also a non-significant main effect of group on P1 choice \((F_{2,42} = .130, p = .878)\); P2 choice \((F_{2,42} = 1.10, p = .344)\); and P3 choice \((F_{2,42} = 2.84, p = .070)\). There was a non-significant interaction between time and group on P1 choice \((F_{2,42} = 1.00, p = .376)\); P2 choice \((F_{2,42} = 2.79, p = .073)\); and P3 choice \((F_{2,42} = .795, p = .458)\).

It is important to note that baseline rGT performance and gambling-like behaviour were comparable in all three groups (Figure 7). Following the LH procedure, there was a significant reduction in P1 selection for the stress and control condition groups (stress group: from 18.3% ± .021 to 13.5% ± .024, \(t_{26} = 2.55, p = .017\); CC group: from 19.4% ± .029 to 11.8% ± .031, \(t_{7} = 3.17, p = .016\)) and a corresponding reduction in P3 selection (stress group: from 3.50% ± .004 to 1.80% ± .004, \(t_{26} = 4.72, p < .001\); CC group: from 2.50% ± .003 to 0.06% ± .003, \(t_{7} = 12.2, p < .001\)) (Figures 9, 11). For the no-stress group, there was a non-significant change in P1 (\(t_{9} = .708, p = .016, p = .497\)) and P3 choices (\(t_{9} = .941, p = .371\)) (Figure 10). Furthermore, there was a significant increase in P2 selection in the no-stress (\(t_{9} = -2.49, p = .034\)) and CC (\(t_{7} = -2.24, p = .049\)) groups; an increase that was not statistically significant in the stress-rats (\(t_{26} = -1.62, p = .118\)).

The significant interaction of time and group on P4 choice suggests that the groups are changing P4 selection over time; however, in different ways (Figure 12). In other words, the
effect of the LH paradigm on P4 selection varied between the groups. To further interpret this, a follow-up univariate ANOVA (difference in pre- vs. post-P4 choice as the dependent variable; group as the independent factor) revealed a significant main effect of group ($F_{2,42} = 7.07, p = .002$). Pairwise comparisons revealed that the stress group significantly increased P4 selection after the LH paradigm more than the no-stress (mean difference: $.094, p < .001$) and the CC (mean difference: $.022, p = .032$) groups (Figure 12). Moreover, the no-stress group significantly decreased P4 selection compared to the stress (mean difference: $-.094, p = .001$) and CC (mean difference: $-.075, p = .024$) groups. Specifically, the stress group significantly increased P4 selection after the LH procedure ($t_{26} = -2.38, p = .025$); on the other hand, the no-stress group significantly decreased P4 selection after receiving one session of escapable footshocks ($t_9 = 2.61, p = .028$).

In order to look specifically at the P2 selection of the rats that underwent the LH paradigm, a separate 2 x 2 factorial repeated measures ANOVA was conducted comparing the P2 selections of the stress and no-stress groups only. The results revealed a significant interaction between time and group ($F_{1,35} = 5.02, p = .035$). This suggests that, while both groups increased P2 selection following the LH paradigm, the no-stress group exhibited a significantly larger increase than the stress-rats.
Figure 9. rGT decision-making of the stress group.

After exposure to inescapable stress, the stress group (n=27) showed a significant impairment in rGT performance. There was a significant increased choice of the riskiest option (P4: $t_{26} = -2.38$, $p = .025$); and significant decreases in options P1 ($t_{26} = 2.55$, $p = .017$) and P3 ($t_{26} = 4.72$, $p < .001$). There was a non-significant increase in the optimal choice (P2) ($t_{26} = -1.62$, $p = .118$) as well. *Indicates a significant difference ($p < .05$). Paired sample $t$-test was conducted comparing Pre- vs. Post-stress data after achieving a significant repeated measures ANOVA. Error bars represent the standard error of the mean for each group.
Figure 10. rGT decision-making of the no-stress group.

Exposure to escapable footshocks resulted in a significant improvement on rGT performance for the no-stress rats (n = 10): there was a significant increase in the optimal choice (P2) ($t_9 = -2.49, p = .034$); along with a significant decrease in the riskiest option (P4) ($t_9 = 2.61, p = .028$). The single session of escapable stress did not have significant effects on P1 or P3 preferences ($t_9 = .708, p = .497, t_9 = .941, p = .371$, respectively). *Indicates a significant difference ($p < .05$).

Paired sample $t$-test was conducted comparing Pre- vs. Post-stress data after achieving a significant repeated measures ANOVA. Error bars represent the standard error of the mean for each group.
Figure 11. Reassessment of rGT performance of the Control Condition Group.

CC rats \( (n = 8) \) only exhibited a significant increase in P2 option \( (t_7 = -2.24, p = .049) \) along with a significant decrease in P1 \( (t_7 = 3.17, p = .016) \) during the second round of rGT testing. There were no other significant changes observed. Data are represented as the mean percent choice of each option. *Indicates a significant difference \( (p < .05) \). For each group, paired sample \( t \)-tests were conducted comparing Pre- vs. Post-stress data after achieving a significant repeated measures ANOVA. Error bars represent the standard error of the mean for each group.
Figure 12. Comparison of P4 selection between the stress, no-stress, and CC groups.
The results revealed a significant interaction between time and group ($F_{2,42} = 7.073, p = .002$) on P4 selection, which suggests that the groups are changing P4 selection over time; however, in different ways. As displayed, both the stress and CC group exhibited an increase in P4 selection after the LH task; however, this increase reached significance in the stress group only ($p = .025$). The no-stress group, on the other hand, decreased P4 selection. Pairwise comparisons revealed that stress rats ($n = 27$) significantly increased P4 selection after the LH paradigm and this increase was significantly higher than both the no-stress ($n = 10$) and CC ($n = 8$) rats. Pairwise comparisons were conducted after a significant interaction of time x group from the repeated measures ANOVA.
3.4.1 Effects of stress on premature responses (i.e. impulsive action)

In addition to impulsive choice/decision-making, the rGT measures premature responding (i.e. impulsive action). The comparisons of pre- and post-stress premature responding of the three groups are displayed in Figure 13. As illustrated, inescapable and escapable footshocks did not significantly alter premature responding ($t_{26} = 2.36, p = .973$ and $t_9 = 1.28, p = .504$, respectively). However, the control condition group significantly decreased the number of premature responses during the second round of rGT testing ($t_7 = -2.45, p = .042$).
Figure 13. Premature responding of the LH, LH control, and control condition groups.
The number of premature responses for the three groups before and after the stress paradigm is displayed. The LH (n = 27) and LH control (n = 10) groups did not exhibit a significant change pre- and post-stress procedure (p > .05); however, the control condition group (n = 8) significantly decreased the number of premature responses during the second rGT testing (p < .05).
3.4.2 Effects of stress on high vs. low impulsivity rats’ P4 selection

Considering the non-significant effect of group and the significant within-subject effect of time on rGT performance, I speculated whether baseline impulsivity levels influenced rGT performance. Therefore, the pre- and post-stress P4 choice (i.e. the most impulsive option) preference of the high impulsivity (HI) and low impulsivity (LI) rats was compared (Figure 14). A 2x2 factorial repeated measures ANOVA (between-subject factor: ‘impulsivity levels’; within-subject factor: ‘time - pre- and post-LH task’) for P4 choice was performed. The results revealed a significant interaction between time and impulsivity levels (\(F_{2,42} = 7.12, \ p = .002\)) and a significant main effect of impulsivity (\(F_{2,42} = 18.7, \ p < .001\)) on P4 choice. Following the LH procedure, the HI rats exhibited a larger increase in P4 selection than the LI rats (mean difference: .180, \(p < .001\)). Next, a 3-way repeated measures ANOVA (between-subject factors: ‘group & impulsivity levels’; within-subject factor: ‘time - pre- and post-LH’) was conducted to compare the P4 selections between the stress and no-stress groups. The results revealed a significant 3-way interaction between time, group, and impulsivity levels (\(F_{2,31} = 5.07, \ p = .012\)), a significant interaction of group and impulsivity (\(F_{2,31} = 7.22, \ p = .003\)), and a significant main effect of impulsivity (\(F_{2,31} = 11.1, \ p < .001\)) and group (\(F_{1,31} = 7.26, \ p = .011\)) on P4 choice.

Prior to the LH task, HI rats significantly favoured the high-risk option more and the optimal option less than the LI rats (see Figure 5 for comparisons). After the stress paradigm, the HI rats from the stress group (HI-stress), that underwent LH procedure, exhibited a significant increase in P4 selection (from 22.2% ± .027 to 35.2% ± .030, \(t_6 = -2.72, \ p = .006\)) (Figure 14). On the other hand, the HI rats from the no-stress group (HI-no-stress), that only underwent escapable footshocks, did not significantly alter P4 choice preference (from 8.10% ± .041 to 4.20% ± .046,
$t_5 = 3.72, p = .530$). Additionally, the LI rats from the stress group (LI-stress) did not alter P4 selection significantly (from 2.20% ± .017 to 3.30% ± .019, $t_{16} = 1.50, p = .912$). However, the LI rats from the no-stress group (LI-no-stress), that only underwent escappable footshocks, showed a significant decrease in P4 selection (from 4.90% ± .032 to 1.20% ± .035, $t_5 = 2.42, p = .006$).
Figure 14. Effects of inescapable and escapable footshocks on high impulsivity (HI) and low impulsivity (LI) rats’ P4 selections.

After exposure to inescapable footshocks, the HI-stress rats \((n = 7)\) had a significant increase in P4 selection. The HI rats that only underwent escapable footshocks (HI-no-stress) \((n = 3)\), on the other hand, did not exhibit any significant change in P4 selection. The LI rats that underwent inescapable footshocks (LI-stress) \((n = 17)\) did not exhibit any change in P4 preference; however the LI rats that underwent escapable footshocks (LI-no-stress) \((n = 5)\) exhibited a significant decrease in P4 selection.
Chapter 4: Discussion

In agreement with previous findings using the rGT (Zeeb et al., 2009), this study demonstrated that, prior to stress exposure; rats demonstrated the capacity to learn the task contingencies of the rGT and discriminate between the advantageous and disadvantageous options. The optimal strategy was to avoid the high-risk/impulsive option (P4) associated with larger rewards but also larger long-term losses and to favour the optimal option (P2) associated with smaller rewards but a larger net food gain. Rodents started out by exploring each option since the reinforcement contingencies were unknown at first. Through trial and error and repeated sampling, rats became aware of the long-term reward/losses associated with each option and eventually developed a stable decision-making strategy. The pre-stress (i.e. baseline) choice behaviour of the rats indicated that they were capable of developing an optimal strategy when choosing between multiple options associated with ambiguous probabilities and magnitudes of reward and loss. This baseline pattern of choice behaviour was stable throughout testing. Of note, humans also present a similar stable pattern of performance on the IGT (Cavedini et al., 2002).

While the majority of rats in the current experiment demonstrated preference for the optimal rGT option, a small subset of them showed a consistent preference for the most impulsive option, suggesting that there are individual differences between healthy rats in choice impulsivity and decision-making. Similarly, a recent meta-analysis that assessed data collected from the rGT between 2008 and 2012 reported that high-impulsive rats, which were grouped based on motor impulsivity (another aspect of impulsivity measured by the rGT), have a higher preference for the disadvantageous rGT option, are slower to learn the task contingencies, and are faster at responding than low-impulsive rats (Barrus et al., 2014). It has been suggested that these poor decision-makers are unable to optimally consider the long-term consequences of their choices.
and are typically driven by an increased sensitivity and motivation for big immediate rewards (Rivalan et al., 2009). This pattern of impulsive decision making, hypersensitivity to rewards, and risk-taking displayed by some healthy humans and rodents, is also observed in PG (Cavedini et al., 2002; de Ruiter et al., 2009) and MD (Must et al., 2006). This suggests that within a healthy population, impulsive decision-making and hypersensitivity to rewards could represent a common risk factor for the development of PG and MD.

In support of our predictions, exposure to acute stress significantly altered subsequent decision-making. However, much to our surprise, exposure to inescapable and escapable shocks had opposing effects on subsequent decision-making. Exposure to the LH procedure resulted in a significant increase in the selection of the most impulsive and high-risk option. These rats did not change preference for the other options, indicating that this type of stress strictly increased preference for the high-risk option. Unexpectedly, exposure to one session of escapable shocks improved decision-making and resulted in a significant increase in the selection of the optimal and a significant decrease in the selection of the most impulsive options. In the no-stress rats, escapable shocks fully shifted choice selection from the most impulsive to the optimal option since there was no significant change in the selection of the remaining options. These observations appear to reflect the improvement and impairment in decision-making following exposure to footshocks, depending on whether or not the animals are allowed to escape it.

Baseline levels of impulsivity also affected rGT performance. High impulsivity (HI) rats from the stress group (HI-stress), that underwent the LH procedure, showed a significantly increased preference for the most impulsive choice; while, the HI rats from the no-stress group (HI-no-stress) that were not exposed to the LH procedure, did not exhibit this shift. The low impulsivity (LI) rats from the stress group (LI-stress) did not alter preference for the risky option; however,
the LI rats from the no-stress group (LI-no-stress) that were not exposed to the LH procedure displayed a significant decrease preference for the most impulsive option. These results suggest that, despite the type of shock, baseline impulsivity also influenced decision-making. Unlike the HI rats, LI rats subjected to the LH procedure did not increase impulsive decision-making. Taken together, both depressive-like behaviours and impulsivity influenced gambling-like decision-making and these effects can be modulated by stress.

4.1 Effects of stress-induced depressive-like behaviour on decision-making

4.1.1 Effects of inescapable shocks on decision-making

Rodents exposed to the LH paradigm (i.e. exposure to inescapable and subsequently escapable footshocks) had difficulties learning to avoid the escapable footshocks. Although the difference in the number of escape responses between the stress and no-stress group was not statistically significant, this is to be expected given that the no-stress group only received 15 sessions of escapable shocks. However, it is apparent that the stress group that underwent the LH procedure was slower to learn to avoid the shocks than the no-stress group. Studies using the LH paradigm have shown that increasing the number of escapable sessions exaggerates the difference in the number of escape responses between the two groups. As shown, the no-stress group was learning to avoid the shocks quicker than the stress group and if they receive additional escapable sessions, this difference is expected to reach statistical significance. Moreover, the main objective of our study was not to specifically observe a statistically significant LH effect between the stress and no-stress groups. The target group of our study was the stress group that underwent the LH procedure and this group clearly showed escape deficits. The no-stress group, on the other hand, only served as a control group for the LH task.
As mentioned, complementing our predictions, the group of stress rats displayed a significant increased preference for the most impulsive option. Further reinforcing this notion, exposure to inescapable footshock did not significantly alter premature responding (i.e. impulsive action) in these rats. Although impulsive action is not directly related to impulsive decision-making, it is another form of impulsivity marked by the inability to withhold an inappropriate response. Impulsive action is highly related to levels of general locomotor activity. Therefore, the observed increased preference for the impulsive option is not a result of general motor hyperactivity and is therefore likely due to the effects of inescapable shocks.

Pre-clinical research on the direct effects of stress on decision-making in animals has been quite limited and often conflicting. One study demonstrated that exposure to chronic and unpredictable stress during adolescence in rodents negatively impacts aspects of decision-making (Chaby et al., 2013), affects risk-assessment (Wang et al., 2014), and increases impulsive choice during adulthood. Chaby et al. (2013) showed that, compared to controls, adolescent-stressed rats are quicker to abandon an incorrect first choice associated with inaccessible food reward and to switch their choice to obtain immediate reward. This decreased latency to correct a choice after an incorrect decision demonstrated in stressed rats was suggested to be the result of decreased behavioural inhibition or increased impulsivity (Chaby et al., 2013). Moreover, chronic stress during adolescence is found to induce a long-term negative cognitive bias, which consequently increases sensitivity to loss and affects the interpretation of ambiguous stimuli (Chaby et al., 2013). Complementing these reports, a recent study reported that rats subjected to chronic exogenous corticosterone during adolescence exhibit increase impulsive choice and decision-making marked by an increased preference for small-immediate rewards rather than larger-delayed ones as assessed by a delayed-discounting task (Torregrossa et al., 2012).
Contrary to the effects chronic stress during early life, pre-clinical research on the effects of acute stress on decision-making has been somewhat conflicting. One study, which investigated the effects of acute stress (i.e. one hour of restraint) on cost/benefit decision-making in rodents using a delay-discounting task, reported that acute stress did not alter preference between the small/immediate vs. the larger/delayed rewards (Shafiei et al., 2012). In another investigation, Koot et al. (2013) assessed the effects of corticosterone injections 30 minutes (rapid) and 180 minutes (delayed) prior to performance on a risk-based decision-making task using the rIGT (a variant version of the rGT). It was reported that, control rats improve performance on the rIGT by decreasing the number of visits to the high-risk disadvantageous arm and shift their choice preference toward the long-term advantageous arm overtime. However, rats subjected to rapid infra-limbic infusions with corticosterone fail to demonstrate this improvement in performance and consistently prefer the disadvantageous arm. This impairment in performance was not observed in rats subjected to delayed corticosterone administration. Interestingly, corticosterone administration did not alter preference for the empty/sham arms, which indicates that rapid corticosteroid injections only impaired reward-related decision-making (Koot et al., 2013). We did not expose our rats to chronic stress; however, our results showing increased choice impulsivity after exposure to acute inescapable shock are in accordance to the results reported by Koot et al. (2013). Thus, it can be concluded that inescapable shocks induced a seemingly risk-proneness profile in these animals and impaired the optimal use of choice strategy.

Despite conflicts in pre-clinical research on stress and decision-making, early clinical findings generally report that exposure to acute stress increases risky behaviour and impair reward-motivated decision-making under uncertainty in the human population (Preston et al., 2007; Starcke et al., 2008).
4.1.2 Possible mechanism of action/ role of stress in increased proneness for risky decision-making

The increased proneness for impulsive decision-making demonstrated in the stressed rats of our study add to a growing body of early clinical evidence finding impulsive decision-making and increased risk-taking following acute stress exposure. Likewise, clinical studies have reported impulsive decision-making in healthy subjects submitted to acute stress (Preston et al., 2007; Starcke et al., 2008), pathological gamblers (Brand et al., 2005; Cavedini et al., 2002; Goudriaan et al., 2005), and depressed individuals (Must et al., 2006; Cella et al., 2010). It is unclear exactly how stress impacts subsequent decision-making. It is known, however, that key brain regions implicated in decision-making overlap with those that are involved with stress reaction and are therefore sensitive to stress-induced changes.

Based on the current clinical and some pre-clinical literature, we suggest a few postulations that may explain how stress can affect subsequent decision-making. It is plausible that stress may alter the underlying mechanisms and processes that are necessary for optimal decision making, such as disrupting the ability to acquire an optimal strategy using previous feedback; or altering sensitivity to rewards and punishments.

The importance of feedback processing in ambiguous decision-making has been highlighted in previous clinical and pre-clinical reports. Optimal performance on both the rGT and IGT requires subjects to use feedback from previously-selected options to guide their choice behaviour. So, it is possible that stress may disrupt feedback processing which in turn impairs the ability to develop an optimal decision strategy. In support for this notion, chronic stress in rodents has been reported to disrupt decision-making by impairing the ability to select appropriate actions on the basis of previously-learned outcomes and the ability to shift to goal-directed behaviour. Dias-Ferreira et al. (2009) reported that rodents exposed to chronic stress
exhibit degradation of the dorsomedial striatum and medial PFC, which was accompanied by an impaired ability to perform actions based on the consequences of their previous choices. Stressed rats become insensitive to previous outcomes and instead shift to habitual response strategy by responding to reward cues that were already learned prior to stress (Dias-Ferreira et al., 2009).

Consistent with the aforementioned pre-clinical findings, clinical studies show that stress (i.e. threat of electric shocks) reduces reward learning in healthy subjects and disrupts their ability to modulate choice behaviour as a function of feedback history (Bogdan et al., 2011). Stressed subjects, who were threatened to receive uncontrollable shocks, show reduced reinforcement learning, which consequently impairs their ability to develop a choice strategy toward a more frequently rewarding stimulus (Bogdan et al., 2011). Similarly, anticipatory stress impairs performance of healthy individuals on a decision-making task similar to the IGT (i.e. Game of Dice) and increases the selection of the disadvantageous option when feedback is provided (Starcke et al., 2008). The anticipatory stress effect is diminished when feedback is not provided; suggesting that anticipatory stress likely impairs feedback processing. Deficits in feedback processing may result in reduced use of negative feedback as a cue to shift to non-risky options and in turn impair reinforcement-guided decision-making (Brand et al., 2004; 2008). Therefore, based on the pre-clinical and clinical evidence it is possible that acute stress negatively impacts the formation of new stimulus-reward associations, which may increase habitual response strategy over goal-directed actions (Dias-Ferreira et al., 2009; Bogdan et al., 2011).

It is also plausible that stress affects reward-related processing, which play an important role in reward-based decision-making. Stress has been found to enhance reward sensitivity and appetitive behaviours such as drug or food intake (Shaham et al., 2000; Sinha, 2008; Lemmens et al., 2011) and gambling (Ledgerwood & Petry, 2006). Human and animal literature suggests that
stress increases these addictive behaviours by altering functions of the reward-related neural networks (reviewed in Koob, 2008). Corticosteroids released during stressful situations have been linked to modulate dopamine activity within the ventral, especially the nucleus accumbens and the dorsal striatum (Barrot et al., 2000; Cabib & Puglisi-Allegra, 2012; Sesack & Grace, 2010), along with the PFC (Salamone et al., 2007); regions that are implicated in incentive reward processing and motivated behaviours (Knutson et al., 2005; Kuhnen and Knutson, 2005). Footshock in rodents, for instance, has been found to increase dopamine secretion within the striatum and medial prefrontal cortex (Abercrombie et al., 1989). This increase in dopaminergic signalling within the striatum in response to acute stress has been linked to increased reward-seeking in rodents (Shaham & Stewart, 1995; Mather & Lighthall, 2012). Stress-induced modulations within these brain regions may therefore increase sensitivity to rewards, promote risk-taking, and increase preference for impulsive options associated with immediate potential gains, regardless of the negative long-term consequences. Consistent with this notion, high cortisol responder individuals, in both humans and animals, are more sensitive to immediate rewards than low cortisol responders (Newman et al., 2007; Van den bos et al., 2009). For instance, Van den bos et al. (2009) reported that acute stress in humans impairs IGT decision-making and promotes risk-taking behaviour in high cortisol responders but not in low cortisol responders.

With reference to both pre-clinical and clinical findings, we may infer that stress interferes with decision-making by disrupting the ability to optimally use feedback to guide future decisions and altering reward-related processing, which may increase sensitivity to immediate rewards and promote disadvantageous choices.
4.1.3 Effects of escapable shocks on decision-making

To the best of our knowledge, the effect of escapable shocks on subsequent decision-making has not yet been investigated. Based on the current results, one session of escapable shocks improved decision-making and decreased impulsive choice. This improvement was certainly unexpected and seems counterintuitive at first; however, it has been previously demonstrated that prior exposure to escapable shocks improves behavioural and physiological deficits caused by inescapable shocks.

This phenomenon has been widely studied based on the learned helplessness theory. It is consistently reported across a number of species that exposure to inescapable and escapable stressful events results in different behavioural and neurochemical outcomes. Specifically, exposure to inescapable footshocks disrupts subsequent behavioural functions such as avoidance learning; whereas exposure to identical but escapable shocks blunts such deficits (Overmier & Seligman, 1967; Cabib & Pugsili-Allegra, 1994; Williams & Maier, 1977; Amat et al., 2010). For instance, dogs exposed to inescapable shocks are unable to learn to avoid future escapable stressors; such impairment is not observed in dogs with previous exposure to escapable shocks (Overmier & Seligman, 1967). Similarly, rodents with prior exposure to inescapable tailshocks show escape response deficits; whereas, previous exposure to escapable tailshocks blunts escape deficits (Amat et al., 2010).

Dhabhar and McEwen (1997) also demonstrated that, upon exposure to acute stress, rats exhibit an increase in immune surveillance in organs that are vulnerable to stress, such as the skin and the brain. This enhancement of the immune response is a defense mechanism intended to protect the organism from a potential future injury caused by a stressful situation (Dhabhar & McEwen, 1997). Rodents exposed to predator odour (acute stressor) enhance lymphocyte
recruitment to the brain, which is associated with reduced anxiety and enhanced ability to cope with the same stressor in a future challenge (Lewitus & Schwartz, 2009).

Similar effects have been observed when animals are able to have a degree of control over the stressful events. In fact, it has been demonstrated that the degree of controllability or predictability of stressors largely affects the behavioural and physiological consequences of the stressful events (Seligman & Maier, 1967). The importance of stressor controllability has been demonstrated using a shocked-yoked design. In this design one animal is subjected to a series of shocks that they are able to terminate or escape by means of an instrumental response. A second animal is yoked to the first animal but it cannot control the shocks on its own. Since the pair is yoked, whenever the first animal terminates the shock, the shock is also terminated for the second animal. So, the two animals receive the same exact shock but one has control over it while the shocks appear unpredictable and uncontrollable to the other animal. Pre-clinical evidence on stressor controllability demonstrate that having a degree of control over stress has shown to reduce the behavioural and neurochemical effects of the stressful exposure; whereas, the lack of perceived control intensifies the stress response (Weiss et al., 1981; Seligman & Maier, 1967).

Furthermore, difference in coping strategies to stress has shown to enhance resilience or vulnerability to stress-induced depressive-like behaviours. Wood et al. (2010) investigated individual differences in coping strategy to chronic social stress in a healthy population of rodents using a resident-intruder model of defeat (i.e. a rodent model of social stress). It was reported that the subgroup of healthy rats that consistently exhibited rapid subordination/submissive posture (i.e. passive behaviour) during the attack (i.e. stress exposure), develops depressive-like behavioural and neurochemical phenotypes (Wood et al., 2010). On the other hand, the subgroup of rats that exhibited upright posture (i.e. proactive behaviour) during
stress exposure is resistant to such depressive-like behavioural deficits. These findings imply that behavioural strategies utilized during stress exposure (i.e. active vs. passive coping) could enhance resilience or vulnerability to stress-induced behavioural depression, respectively. In a similar investigation, Krishnan et al. (2007), demonstrated that a subset of inbred mice exposed to chronic social stress, display depressive-like behaviours such as, decreased social interactions and sucrose preferences (i.e. susceptible mice), while the rest of them do not exhibit such deficits (i.e. resilient mice). It was further reported that the susceptible mice exhibited increased activity in the VTA dopamine neurons compared to the resilient mice; a region which is generally implicated in promoting reward-related behaviours (Berridge & Robinson, 2003).

Based on the limited pre-clinical evidence on stress resilience, we suggest that, previous exposure to escapable shocks may help organisms develop improved and more effective strategies to cope with future mental challenges such as decision-making by increasing vigilance or sensitivity to losses (Maier & Watkins, 2010).

### 4.2 Effects of baseline impulsivity on decision-making

Considering the unexpected improvement in decision-making of the no-stress group, we speculated whether baseline impulsivity had a modulating influence on the effects of shock exposure. As pointed out, rats displayed individual differences in baseline impulsivity where a subset of them showed a consistent preference for the most impulsive option. Following the LH paradigm, the HI rats that were exposed to inescapable shocks (HI-stress rats) showed a significant increase in the selection of the most impulsive option. Interestingly, the HI rats from the no-stress group (HI-no-stress) that only underwent escapable shocks did not significantly alter preference for the most impulsive option. The LI rats from the stress group (LI-stress), in contrast, did not significantly change preference for the impulsive option; however, the LI rats
from the no-stress group (LI-no-stress) showed a significant decreased preference for the most impulsive option. This is perhaps this is the most novel and unexpected findings of our study. To the best of our knowledge, no other study has concurrently assessed the effects of baseline impulsivity and stress-induced depressive behaviours on impulsive decision-making. Based on our results, baseline impulsivity seemed to modulate the behavioural effects of shock exposure. Upon exposure to inescapable shock, HI rats increased preference for the risky option; whereas LI rats did not alter preference. In other words, high levels of baseline impulsivity in addition to stress-induced depressive behaviours (i.e. HI-stress rats) increased impulsive decision-making. Whereas low levels of baseline impulsivity in addition stress-induced depressive behaviours (i.e. LI-stress rats) did not increase impulsive decision-making. Moreover, escapable shocks only improved decision-making in rats with low levels of baseline impulsivity.

4.3 Reassessment of rGT performance of rats in the control condition group

Another unexpected finding from our study was the reassessed rGT performance of the control condition rats. To the best of our knowledge no study has reassessed the rGT performance of the same rats. As previously stated, these rats significantly increased selection of the optimal option during the second round of rGT testing. Importantly, there was no change in the selection of the risky option, suggesting that there was a significant improvement in rGT performance. Interestingly, this group also made significantly fewer premature responses during the second round of rGT testing compared with the stress and no-stress groups. We certainly did not expect this significant improvement since performance on the rGT is considered a fairly stable trait. One possibility is the effects of learning where there might be a continuous improvement on learning the task contingencies and decision-making strategy.
4.4 Relationship between stressed-induced depressive behaviours, impulsivity, and pathological gambling

Based on the foregoing review of what is known in the literature, an association between pathological gambling, depression, and impulsivity has been consistently demonstrated. High impulsivity traits positively correlate with gambling severity (Alessi & Petry, 2003; Steel & Blaszczynski, 1998). Additionally, impulsive traits and depressive symptoms both positively and independently correlate with gambling severity (Turner, Jain, Spence, & Zangeneh, 2008; Kim & Grant, 2001; Blaszczynski and Nower, 2002) and preliminary findings suggest that as levels of depressive symptoms increase, the severity of gambling also increases as a function of impulsivity (Clarke, 2006). Also, the presence of both impulsive traits and depressive symptoms during early adolescence is positively associated with gambling problems during late adolescence (Lee et al., 2011).

In accordance with these findings, impulsive decision-making has been linked to both pathological gambling and depression. PG individuals tend to have difficulties evaluating long-term consequences of choices and display a strong preference for impulsive choices associated with high immediate rewards and neglect the long-term adverse consequences (Cavedini et al., 2002; Brevers et al., 2014). Similar pattern of impulsive decision-making has also been reported in some individuals with depression (Must et al., 2006; Cella et al., 2010). It remains unclear why some individuals are prone to consistently make risky decisions and develop gambling behaviours; perhaps this may be a common underlying phenotype associated with these two illnesses.
4.4.1 Dysfunctional reward pathways in PG and MD

The exact mechanism underlying impulsive decision-making in PG and MD is not fully known. Detailed investigation into the cognitive mechanisms underlying impulsive decision-making in PG has revealed a profile of impaired decision-making similar to patients with vmPFC lesions. PG individuals exhibit abnormal activity within brain regions implicated in ambiguous decision-making such as the ventromedial prefrontal cortex (vmPFC). Some neuroimaging studies report that the reward and motivational system in PG show attenuated sensitivity towards monetary wins and losses along with heightened reward-seeking behaviours (Reuter et al., 2005; de Ruiter et al., 2009). Compared to controls, pathological gamblers exhibit lower ventral striatal and vmPFC activity in response to monetary gains, which is correlated with poor performance on gambling-related decision-making tasks (Reuter et al., 2005). Decreased vmPFC activity during rewarding stimuli as reported in PG individuals indicate a possible deficits in integrating incentive information that may be used as a guide to subsequent decision-making (Balodis et al., 2012).

Interestingly, impaired decision-making and impulsivity are also related and positively correlate. Baladois et al. (2012) reported a positive correlation between diminished VS activity and self-reported impulsivity in PG individuals. Kräplin et al. (2014) similarly reported a positive correlation between impairments in the valuation components of decision-making, as assessed by a gambling decision-making task (i.e. Cambridge Gambling Task), with impulsivity. Impulsivity, therefore, may be an important indicator of the imbalanced reward processing in PG individuals.

Together, increased reward-seeking behaviour, reduced reward sensitivity characterized by hyporesponsiveness in the VLPFC (de Ruiter et al., 2009), VMPFC (Reuter et al., 2005), along with heightened impulsivity in pathological gamblers implicates a blunted neurophysiological
response to rewarding and punishing stimuli and may promote risky decision-making that are tuned to immediate short-term rewards.

Furthermore, alterations in reward and punishment sensitivity may cause misinterpreted valuations of rewards or punishment and suboptimal use of feedback, which can promote risky and reward-seeking behaviour and continued gambling (van Holst et al., 2010). This is in accordance with current addiction models suggesting that individuals with a diagnosis of addiction exhibit reduced dopaminergic transmission within the reward neural networks, which predates the development of addictive behaviour. Consequently, some authors argue that gamblers are likely to seek more rewarding acts to compensate for a pre-existing reward-diminished state (Berridge & Robinson, 1998; 2008). However, it has not been directly demonstrated whether this diminished reward and punishment sensitivity is a consequence or a precursor of addictive behaviours. In fact, recent imaging evidence report normal dopamine receptor levels in the striatal regions of PG individuals and increased DA release in response to stimulant challenge, compared to controls (Boileau et al., 2013; 2014; Clark et al., 2012). As mentioned, these findings are instead consistent with the incentive-sensitization theory of addiction, in which repeated reward seeking alters the brain’s DA neural substrate, making it hypersensitive to the rewards and thereby promoting rewarding behaviours (Robinson & Berridge, 1993).

Due to conflicting findings, the exact underlying mechanism of the reward system in PG is not yet entirely known. From this perspective, gambling behaviours may be caused and maintained, in part, by impaired decision-making resulting from abnormal processing within these brain regions.

Similarly, dysfunction within the reward pathway has been demonstrated in depression (Naranjo et al., 2001). Depressed individuals display general diminished sensitivity to previously
enjoyed activities, termed anhedonia, which is associated with reduced activation in the reward-related brain regions. Specifically, depressed individuals display blunted activation of the striatal (Pizzagalli et al., 2009) and the frontal regions (Knutson et al., 2008) in response to reward-predicting cues. To compensate for this ‘anhedonic state’, individuals may engage in reward-seeking behaviours and favour immediately rewarding options. Nevertheless, not all individuals diagnosed with depression develop addictions and levels of anhedonia are variable amongst those diagnosed with MD. It is possible that additional mechanisms or behavioural traits may influence the vulnerability of individuals diagnosed with depression to develop addiction.

Drawing from our findings, it is possible that the link between depression and addiction could be exacerbated for individuals who are impulsive when experiencing stress or negative mood states.

As demonstrated, HI rats that were exposed to inescapable shocks and exhibited depressive-like behaviours increased impulsive decision-making; in contrast, LI rats faced with the same inescapable shock did not exhibit such impairments. In line with this notion, it has been demonstrated that depressed, impulsive adolescents drink more heavily than depressed, non-impulsive or non-depressed individuals (Hussong & Chassin, 1994). Given the characteristics of high impulsivity, we suggest that impulsive individuals are sensitive to immediate rewards and are more likely to disregard the long-term negative outcomes of their choices in favour of immediate rewards. In response to stress or depressed mood, these impulsive individuals are more likely to have difficulties regulating behaviours, resisting urges, and are likely to engage in impulsive decision-making and immediately-rewarding acts such as gambling, disregarding the long-term consequences.

From this perspective, it is reasonable to postulate that manifestation of anhedonic symptoms such as, general blunted emotional reactivity, altered reward processing, along with impulsive
traits reported in PG and MD, may result in an impaired ability for normative decision-making in the context of rewards. This has recently been coined as ‘decisional anhedonia’ (Treadway & Zald, 2011), which is characterized by the impaired ability to balance costs and benefits among multiple choices. It is suggested that a general anhedonic state may prevent optimal integration of cost/benefit information associated with multiple options, leading to abnormal choice behaviour and impaired decision-making.

4.5 Stress reactivity and increased risk for PG and MD

As reviewed in the introduction, studies of addictions and depression have consistently reported that stress plays an etiological role in the development of these disorders (reviewed in Sinha, 2001). Exposure to acute stress in both humans and animals is associated with increased vulnerability to addictive behaviours, depressive-like behavioural deficits, and risky decision-making (Goeders, 2002; Weiss, 1968; Preston et al., 2007). The existing literature on stress and addiction suggests that stress modulates engagement in rewarding behaviours such as, drug-seeking and gambling, and restores previously extinguished addictive habits (Sinha, 2001). There is emerging evidence that stress activates the reward pathways and enhances meso-cortical dopamine release, which in turn enhances sensitivity to the reinforcing effects of drugs (Mather & Lighthall, 2012; Goeders, 2002; Piazza et al., 1989; Abercrombie et al., 1989). Moreover, both early life and acute stress, particularly those deemed uncontrollable, have been associated with increased risk for depression (Weiss & Simson, 1986; Krishnan et al., 2007; Heim et al., 2004) and anhedonic behaviours such as reduced self-reported ratings of pleasure and reward responsiveness (Berenbaum & Connelly, 1993; Wichers et al. 2009; Bogdan & Pizzagalli, 2006).

The prevailing findings emphasize the prominent role of stress in addiction and depression, which likely arises from dysfunctional interactions between the stress and reward pathways.
Given that MD and PG are both associated with reward-related impairments (Pizzagalli et al., 2009; Bogdan & Pizzagalli, 2006; Reuter et al., 2005; de Ruiter et al., 2009), and that stress increases the risk for addiction and depression (Heim et al., 2004) and modulates brain reward pathways (Bogdan et al., 2011; Bogdan & Pizzagalli, 2006; Knutson et al., 2005; Mather & Lighthall, 2012); it is reasonable to speculate that stress-induced reward processing deficits result in cognitive dysfunctions, such as impaired decision-making, which may, in turn, increase the risk for depression and addiction.

This being said, stress is not sufficient to cause depressive symptoms or addictions per se. It has become increasingly clear that individuals exhibit different behavioural responses to stress. Most individuals do not develop depression or abuse drugs following stressful life events. It is not fully understood why some individuals are resistant to stress-induced psychopathologies. Past clinical studies reported that, compared to healthy individuals, pathological gamblers and drug users exhibit abnormal levels of cortisol, a reliable measure of the stress system, following exposure to rewarding stimuli (Paris et al., 2010; Lovallo, 2006). Depressed individuals also exhibit elevated levels of cortisol following exposure to a stressor (Gold et al., 1986). Consistent with this notion, individual differences in neural responsiveness to rewarding stimuli correlates with resilience to stress-induced depressogenic effects. Nikolova et al. (2012) found stress-induced depressogenic effects (i.e. low positive affect) in subjects with low ventral striatum reactivity to rewards, while those with high VS reactivity are protected against these depressogenic effects.

Combined, these findings suggest that individual differences in stress reactivity and reward pathways may shape one’s propensity to experience cognitive impairments in the face of stress. It is possible that deficits in the regulation of stress could result in abnormal stress responses and
contribute to these illnesses. Impaired stress responses may cause some individuals to become more sensitive or reactive to the depressive impacts of stress than others and may encourage the engagement in rewarding habits, such as gambling, and increase vulnerability to other types of addictions.

4.6 Conclusion

The overall aim of this study was to enhance our understanding of the relevance of stress-induced depressive behaviour on gambling-like decision-making in rodents. Previous literature reports an association between pathological gambling, depression, and elevated impulsive traits. Impulsive pattern of decision-making has been associated with both PG and MD, where there is a significant preference for impulsive options that deliver high immediate rewards despite the long-term negative payoffs. Multiple lines of evidence converge to suggest that stress might increase the risk for depression and gambling by disrupting reinforcement learning, reward processing and responsiveness, all resulting in impaired decision-making which may further contribute to these illnesses. Based on the current literature, we speculated whether depressive-like behaviour induced by acute stress would impair subsequent gambling-like decision-making in rodents. To address this aim, we coupled the LH and the rGT tasks and measured baseline rGT choice behaviour and re-assessed rGT performance following exposure to acute stress. We hypothesized that acute stress would increase impulsive decision-making in rodents.

Drawing on our current results, exposure to acute stress using the LH task increased impulsive decision-making akin to those found in PG and MD. In support of our hypothesis, inescapable footshocks induced depressive-like behaviours in rodents (i.e. escape deficits) and induced a risk-proneness profile marked by an increased selection of the impulsive option that resulted in high immediate rewards. The exact mechanism underlying stress and decision-making is not
clear. Based on the current pre-clinical and clinical literature, we speculated that acute stress interferes with the underlying processes involved with decision-making such as, the ability to optimally utilize feedback to guide future decision-making; and increases sensitivity to immediate rewards associated with impulsive options. Whether these dysfunctions in humans are associated with disruptions in mesolimbic reward circuitry, however, remains unknown.

Much to our surprise, exposure to one session of escapable shocks, improved decision-making. To the best of our knowledge the effect of escapable shocks on decision-making has not been investigated. Based on indirect evidence on stressor controllability and some pre-clinical findings, we speculated that prior experience with escapable shocks may aid in the development of coping strategies to face subsequent challenges.

Moreover, baseline impulsivity modulated the effects of stress on decision-making. We observed that high levels of baseline impulsivity along with stress-induced depressive-like behaviour increased impulsive decision-making; this impairment was not observed in rats with low levels of baseline impulsivity. This is in accordance with preliminary findings in the PG literature, which reports that impulsivity mediates the effect of depressive vulnerability on gambling severity (Clark, 2006). The findings in the present study add to the growing body of literature regarding impulsivity and decision-making that may help elucidate mechanisms underlying the relationship between stress, MD, PG and other types of addiction.

4.7 Clinical implications

Investigating decision-making deficits in individuals with pathological gambling or depression is of crucial importance, however large longitudinal studies are necessary to better
elucidate the relationships between stress, MD and PG. Animal models offer the opportunity to investigate the impact of stress and monitor the development of gambling-like behaviours in a relatively short timeframe. Animal studies that include pre- and post-stress exposure assessments, controlled experimental conditions, and longitudinal assessments, provide valuable information that could not be obtained from human studies. Longitudinal or prognostic investigations of PG in humans require substantial investments in terms of funding and duration. Animal models also enable greater manipulation and control of variables. Quasi-experimental investigations and ethical constraints in humans do not permit similar exploration of causal relationships.

To the best of our knowledge, the direct impact of stress on subsequent decision-making in rodents is lacking. The rGT coupled with the LH task provides a unique opportunity to assess the direct impact of stress on subsequent gambling-like decision-making. With this novel procedure, we have provided direct evidence that inescapable shock increases subsequent impulsive decision-making and gambling-like behaviour in rodents. We have also shown that escapable shocks may improve decision-making strategy and decrease gambling-like behaviour. Similarly, in humans, the precise mechanisms underlying the relationship between stress-induced deficits in decision-making and addiction remain relatively unclear. Further inquiry of the neurobiology of stress and decision-making holds great promise for developing clinical interventions for the prevention and treatment of gambling and depression.

By integrating multiple behavioural paradigms, our work provides an encouraging framework for elucidating the neurobiological factors of impaired decision-making as a potential risk factor for developing addictive behaviours and depression. Understanding the effects of stress-induced depressive behaviours on cognitive processes and impulsivity has clear implications for developing new pharmacological agents and more specific treatment strategies for this group of
individuals. Currently, there are no medications that can be specifically recommended for the treatment of PG.

Based on the current findings, novel clinical efforts regarding treating PG, especially those who gamble to escape depression, could focus on highly impulsive individuals and promote better ways to cope with stress. New and more efficient interventions could focus on improving decision-making habits in the context of stress among at-risk individuals, which may ultimately help reduce susceptibility to develop gambling dependence. The modulatory effect of escapable shock on improving decision-making in rodents observed in this investigation, suggests that such procedure may represent a promising path for treatment.

4.8 Limitations

It could be argued that the impaired rGT performance exhibited in the stress group could arise from the fact that this group received a higher number of stress sessions (i.e. inescapable in addition to escapable shocks) compared to the no-stress group. In other words, their impairment could be due to the accumulation of stress sessions rather than the escapability of the footshocks. This issue was previously addressed to a certain degree. Seligman et al. (1968) investigated whether escapable shocks reverse or counteract the escape deficits following inescapable shocks. In this experiment, dogs with previous experience with inescapable shocks were physically dragged across the shuttle box and were shown that escape is possible during the escapable session. After a number of escapable sessions, the authors reported that the deficits induced by inescapable shocks disappeared after a few escapable shock sessions. This weakens the argument that the number of stress session could result in behavioural deficits since such effects were blocked by a procedure that involved exposing subjects to more rather than less shock.
By the same token, one could argue whether the improvement in rGT performance observed in the no-stress rats persists if they receive additional escapable shocks or even inescapable shocks. Therefore, to strengthen the conclusions from our observations, it would be important to utilize a different task that specifically addresses the controllability of the shock/stress, which would be done by using a shocked-yoked design, explained in the following section.

Furthermore, due to the large variability in the P4 selection of the HI rats, it would be important to try to replicate our results using a larger sample size.

4.9 Future directions

Considering the pre-clinical and clinical literature on the effects of stressor controllability on subsequent behaviour, future studies should aim to assess the effects of uncontrollable and controllable stress on decision-making using a shocked-yoked design. As pointed out, having a degree of control over stress has shown to reduce the behavioural effects of stress exposure, such as avoidance learning (Seligman & Maier, 1967). Considering the current findings, it might be interesting to assess the effect of stressor controllability on subsequent decision-making.

Specifically, two groups of rats would be exposed to footshocks; however, one would be able to control and terminate the shock for both groups; subsequently, the effects of stressor controllability on gambling-like decision-making could be assessed using the rGT. Moreover, more escapable sessions could be added to the LH paradigm in order to exaggerate the difference in escape responses between the two groups.

It might also be interesting to investigate the effects of depressive-like behaviour on decision-making induced by a chronic stress model such as the chronic-unpredictable stress task (CUS). Moreover, in addition to escape deficits, other behavioural measures of depression could be conducted. For instance, the LH task is known to induce other behavioural and neurochemical
changes that resemble human depression such as: modifications of sleep patterns, taste aversions, increased finickiest about drinking quinine-adulterated water, hormonal changes, and decreased food intake. Furthermore, future research may aim to investigate the effects of both acute and chronic stress on other decision-making tasks that assess impulsive choice such as, delayed-discounting and probability-discounting tasks.

Future research is also needed to investigate the molecular and neurobiological substrates involved in the reward system that may mediate stress-induced impairments. Investigating the interface between stress, impulsivity, and decision-making will address an important knowledge gap both in the field of addictions as well as in the field of depression. It is clear that stress modulates the transition to addiction and increases drug-seeking behaviours. Genetic approaches would help identify specific molecules implicated in both stress and reward pathways that may also be implicated in PG and MD. In fact, the current study is part of a larger parallel human genetic association investigation aimed to elucidate the comorbidity between MD and PG. The human phase of this study investigated the correlational relationship between PG, MD, and impulsive personality traits; while the animal phase (i.e. the present study) aimed to address the causal relationship amongst these variables.

Specifically, in the human phase we aimed to assess the simultaneous correlational relationship between PG severity, depressive symptoms, and impulsive traits in humans. To address this objective, a sample of 400 participants, including PG individuals and healthy controls, completed a series of instruments designed to assess these primary variables. Complementing previous studies, our preliminary results indicate that depressive symptoms positively correlated with gambling severity and that high levels of self-reported impulsive personality traits exerts an additive effect on PG severity. Specifically, in a sub-sample (168 PG subjects) depressive symptoms, self-reported impulsive personality traits such as Novelty
Seeking and Self-Directedness, were highly correlated with PG severity. These results seem to complement the findings from the animal phase of our investigation. As reported, stress-induced depressive symptoms increased gambling-like decision-making; this stress effect was worsened when rats had higher levels of baseline impulsivity.

Given the heritability of impulsive traits (Gillespie, Cloninger, Heath, & Martin, 2003) and of PG (Eisen, et al., 1998; Slutske et al., 2010), and the fact that the comorbidity of PG and MD is moderately influenced by genetic factors (Potenza et al., 2005), the next step would be to take into considerations measures of impulsivity and MD in genetic association studies of PG. Specifically, we are conducting a molecular genetic association study to investigate the interaction between these variables (i.e. PG severity, impulsivity, and depressive symptoms) and specific genes involved in the regulation of stress and reward-related processes. Our lab has the largest PG molecular genetic association studies in case-control and sibling-pair samples (Lobo et al., 2007; Lobo et al., 2014) and includes DNA samples of over 700 subjects with the full spectrum of gambling behaviours. Considering the results obtained from our animal phase along with the literature on stress and addictions and depression, we hypothesized that genes involved in the stress and reward systems will be more significantly associated with PG severity in subjects presenting higher levels of depressive symptoms and impulsivity. In other words, in subjects with higher depressive symptoms, polymorphisms in 6 proposed genes are significantly associated with higher PG severity. To address this, 27 polymorphisms for the BDNF, NTRK2, CRH, CRHR1, CREB, and SERT genes of the stress and reward pathways were genotyped. Currently 12 out of the 27 polymorphisms have been analyzed and results indicate significance main effect for the CRH and CRHR1 genes; which are both implicated in stress regulation and reward processing. The next step would be to complete the analysis of every SNP.
Lastly, considering the mixed findings in the current literature, future research should also aim to closely elucidate the neural substrates underlying the interaction between stress responsivity and decision-making using various functional neuroimaging techniques during performance on various decision-making tasks such as the rGT.


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