Abstract

Impulsivity and stress are two of the most important determinants of drug addiction in that both factors predict the initiation and maintenance of drug use, as well as relapse to drug taking following abstinence. Despite this combined influence, the interaction between stress and impulsivity has never been examined systematically in animal models of addiction. The objective of the current study is to examine the role of acute stress on two different measures of impulsivity in rats: the Go/No-go test measures motor impulsivity, and the Delayed Reinforcement Paradigm measures cognitive impulsivity. To determine whether a 1 hr restraint stress is physiologically stressful, blood samples from rats in Experiment 1 were taken at 5 different sampling points: baseline (0 min), reactivity (15 and 60 min) and recovery (100 and 180 min). In Experiments 2 and 3, rats were tested in either the Go/No-go test or the Delayed Reinforcement test immediately following 1 hr of restraint stress. Results from Experiment 1 show that 1 hr of restraint stress increased plasma corticosterone concentrations at 15 min and 60 min; corticosterone concentrations returned to baseline levels by 100 min. Following stress, the percentage of Go interval responding was not altered during Go/No-go testing (Experiment 2), nor were there changes in the indifference point values during Delayed Reinforcement testing (Experiment 3). These results suggest that 1 hr of acute stress does not increase either motor or cognitive impulsivity, and stress may influence addiction via mechanisms that are independent of impulsivity.
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List of Abbreviations

5-CSRTT – 5-Choice Serial Reaction Time Task
AUC – Area Under the Curve
CRF – Continuous Reinforcement
CRF – Corticotropin-Releasing Factor
CRF₁ – Corticotropin-Releasing Factor Receptor Subtype 1
CV – Coefficient of Variation
DRL – Differential-reinforcement of Low Rate Schedules
EPM – Elevated Plus Maze
HPA – Hypothalamic-Pituitary-Adrenal
$S_D$ – Discriminative Stimulus
VI – Variable Interval
Chapter 1
Introduction

Drug addiction is a complex behavioural disorder that is perpetuated by stressful life events and predisposing personality characteristics, most notably impulsivity. Those who become addicted may be more susceptible to reinforcing effects of drugs or to the learned associations related to drug taking (e.g., environmental cues) (Koob & Kreek, 2007; Olmstead, 2006). Furthermore, genetic or physiological influences may increase one’s vulnerability to addiction, by lowering the threshold of drug exposure required to lead an individual from experimental to compulsive drug use (Nestler, Barrot, & Self, 2001; Olmstead, 2006; Piazza & Le Moal, 1996). Together, these vulnerabilities may increase the risk of first-time use as well as maintenance and relapse of drug use. If a vulnerable individual experiences stressful life events, or is highly impulsive, their risk of initiating drug use and maintaining drug taking behaviour increases.

Acute and chronic stress are significant risk factors for the initiation and maintenance of drug addiction, and for relapse to drug use following abstinence (Koob, 2008; Wilsnack et al., 1997). Indeed, individuals who report high levels of perceived stress also report longer periods of cocaine use compared to low-stress controls (Karlsgodt, Lukas, & Elman, 2003). Animal research confirms that acute (one session) and chronic (repeated sessions) stress contributes to all stages of drug-taking behaviour, from drug use to abuse to relapse (Goeders, 1997; Goeders, 2002; Lu, Shepard, Hall, & Shaham, 2003).

The effects of stress on drug intake, in both animals and humans, are mediated through the hypothalamic-pituitary adrenal (HPA) axis system. Drugs of abuse activate
the HPA axis, which facilitates brain reward systems, thereby enhancing drug-seeking behaviour (Goeders, 1997; Piazza, et al. 1993; Sarnyai, Shaham, & Heinrichs, 2001). Moreover, rats with heightened behavioural and neuroendocrine responses to stress acquire psychostimulant self-administration more rapidly than do low responders (Piazza & Le Moal, 1997), whereas inhibiting HPA activity decreases both the acquisition and maintenance of cocaine self-administration (Goeders & Guerin, 1996; Specio et al., 2008). Similarly, human cocaine addicts display atypical HPA responses to stressors, which may contribute to their compulsive drug use (Kreek, 1996; Kreek & Koob, 1998). A stress-induced increase in HPA axis activity is also a strong precipitator of relapse in both humans and animals. In the animal model, physical stressors such as foot shock reinstate cocaine- and heroin-seeking following extinction; pharmacological agents that augment HPA axis activity (e.g., corticosterone and corticotropin-releasing factor agonists) have similar effects (Shalev, Grimm, & Shaham, 2002). The augmentation of corticosterone secretion during cocaine withdrawal (Mantch, et al. 2007) likely explains the increased propensity to relapse in this model.

A completely separate line of research has examined impulsivity in relation to drug addiction. Impulsivity is characterized by several distinct features including intolerance for delayed reward, impaired ability to consider consequences of an action, and behavioural disinhibition (Evenden, 1999). The degree of impulsivity varies across individuals and the lifespan; with high levels of impulsivity making an individual more susceptible to several psychiatric disorders, one of the most problematic being drug addiction (Olmstead, 2006). The two aspects of impulsivity that relate most directly to drug addiction are motor and cognitive impulsivity. Motor impulsivity reflects the
inability to withhold a response, also known as behavioural disinhibition; cognitive impulsivity refers to the inability to delay gratification (Brunner & Hen, 1997). Motor and cognitive impulsivity are two separate types of impulsivity, that may be mutually exclusive (Olmstead, 2006; Winstanley, Eagle, & Robbins, 2006). High baseline levels of either motor or cognitive impulsivity increase the risk of initiating, maintaining and relapsing to drug use in humans and animals (Perry & Carroll, 2008; Perry, Larson, German, Madden, & Carroll, 2005). More specifically, humans who abuse cocaine or heroin prefer smaller, immediate rewards over larger, delayed rewards (both drug and non-drug) and display impaired inhibition on tasks measuring motor impulsivity (Fillmore & Rush, 2002; Kirby & Petry, 2004; Li, Milivojevic, Kemp, Hong, & Sinha, 2006). Similarly, on measures of both motor and cognitive impulsivity, high impulsive rats display greater drug-seeking behaviour than low impulsive rats, suggesting that impulsivity influences drug taking behaviour (Belin, Mar, Dalley, Robbins, & Everitt, 2008). Furthermore, impulsivity predicts the acquisition, escalation, and reinstatement of cocaine taking-behaviour in that high impulsive rats display greater drug-taking behaviour than do low impulsive rats (Dalley et al. 2007; Perry et al., 2005; Perry, Nelson, & Carroll, 2008).

Despite extensive evidence that stress and impulsivity are significant contributors to drug addiction, few studies have examined the relationship between these two processes. The aim of this research is to test the hypothesis that acute stress increases cognitive and motor impulsivity in animal models. To do so, rats underwent restraint followed by impulsivity testing in either a Go/No-go or Delayed Reinforcement task, which measure motor and cognitive impulsivity, respectively. This research will begin to
elucidate the possible relationships between stress and impulsivity and will lay the groundwork for the subsequent evaluation of stress-impulsivity interactions on drug-seeking and drug taking in rats.
Chapter 2
Methods

Subjects

Male Long Evans rats ($N = 60$; Charles River, PQ, Canada) weighing 275-300 g at the start of the experiments were tested. Rats were housed in a temperature and humidity controlled room under a 12 h light/dark cycle with lights on at 1900 h. Animals were housed in pairs in Plexiglas cages ($40.0 \times 25.0 \times 22.0$ cm) containing wood chip bedding (Beta Chip; Northeastern Products Corp., NY) and one polycarbonate plastic tube. Water was available ad libitum. Following one week of habituation to the colony room, during which time food was available ad libitum (Lab Diet, PMI Nutrition International, Inc.), animals were fed for 90 min each day. Animals used for pilot testing and for the three experiments were food restricted, and housed in the environment described above. Animal care was conducted in accordance with the Canadian Council on Animal Care and the experiments were approved by the Queen’s University Animal Care Committee regulations.

Apparatus

Operant Chambers. Training and testing took place in operant chambers ($26.5 \times 22.0 \times 20.0$ cm) each containing a sound attenuating box (built in house). Boxes are fitted with a ventilator fan providing low-level background noise. On one wall, two retractable levers are positioned equidistantly on either side of a food magazine. Levers are 3.5 cm wide, 5.0 cm from the metal grid floor (3 mm in diameter, spaced 11mm apart) and 12.0 cm apart. The pellet dispenser (Med Associates, VT) delivers sugar pellets (45mg, Bio-
Serv, NJ) to the food magazine; infrared sensors detect food collection and nose pokes within the magazine. Cue lights above each lever and the food magazine are mounted 10 cm from the grid floor. A house light is positioned on the outside of the operant chamber. The equipment is controlled, and data are collected by an IBM-type computer located in an adjacent room with software programs written in house.

*Elevated Plus Maze.* The elevated plus maze (EPM) is a plus-shaped maze constructed of urethane-sealed wood. The maze is raised 50 cm above the floor, with two of the opposing arms (50 × 10 cm) being enclosed by 40 cm walls (closed arms), and the remaining two arms (50 × 10 cm) having no walls (open arms). Each arm is at a 90° angle to the adjacent arm. EPM testing was performed in a room illuminated by a red light, recorded using a digital video camera (Sony DCR-HC46), and coded using Observational Data Logging (OD Log; Macropod Software).

*Restraint Stress.* Restraint stress was administered prior to impulsivity testing. Rats were individually placed in Plexiglas cages (40.0 × 25.0 × 22.0 cm) containing sawdust bedding and transferred to the room where the restraint stress occurred. Stressed rats were restrained in plastic DecapiCone restrainers for 60 min, head excluded, in the prone position. To reduce mobility, two water bottles were placed on either side of the restrained rat. A light (60 w) was positioned approximately 2 ft above the rat cage. The stress manipulation was completed in a room adjacent to the operant chambers. Control animals were individually placed in identical Plexiglas cages containing one polycarbonate tube and moved to an adjacent room for 60 min.
Procedure

Pilot Testing. To verify that restraint stress induces behavioural effects, a separate group of rats (N = 12) were tested on the EPM following 60 min of restraint stress as described above. Prior to test day, rats were habituated to the testing room for 20 min on three consecutive days. On the day of testing, rats were placed on the maze facing a closed arm, and allowed to explore the maze for 5 min. Arm entry was defined as all four paws in a given arm. Behaviours measured were percentage of time spent in the open arms and percentage of entries into the open arms. If a rat fell off an open arm, it was returned to the center of the maze. The maze was cleaned after each animal using 70% ethanol.

Experiment 1: Corticosterone Reactivity. To verify that the restraint stress manipulation activates the HPA axis and induces a hormonal stress response, blood was collected and assayed for plasma corticosterone in a group of stress and control rats (N = 10 each). On the day prior to restraint and blood sampling, both hind legs of each rat were shaved. Rats were gently held and whole blood samples were collected from the saphenous vein into non-heparinized tubes (500 µL), at which point the samples clotted for 60 min. Following clotting, samples were centrifuged at 2800 × g for 30 min to separate plasma from red blood cells. Plasma was stored in 500 µL Eppendorf tubes at -20 °C until corticosterone concentrations were determined by a rat corticosterone enzyme immunoassay kit (Active Rat Corticosterone EIA, Diagnostic Systems Laboratories, Inc., Texas). Corticosterone assays were performed in Dr. Wynne-Edwards’ laboratory at Queen’s University, Kingston, Ontario, Canada. The corticosterone analyses were
performed in accordance to the manufacturer’s instructions. Whenever possible, samples were quantified in duplicate.

For three consecutive days prior to testing, rats were habituated to the blood sampling room, and the room where they were kept during stress testing for 30 min. During habituation and testing, animals were kept in individual cages. During testing animals remained in their respective rooms until the final blood sample was taken, at which point the animals were moved back to the colony room. Samples were taken from the rats immediately prior to restraint (baseline; 0 min), 15 min following the initiation of the restraint, immediately following the termination of the restraint (60 min), and 40 min and 2 hr following the restraint. The blood samples taken 15 min into the stressor and immediately following the stressor measure stress reactivity. Samples taken at the 40 min and 2 hr post-stress periods measure stress recovery. Blood samples were collected from control rats at the same time points.
Experiment 2: Go/No-go. Behavioural disinhibition was measured using a Go/No-go task, based on procedures described by Paine, Dringenberg, and Olmstead (2003) (Figure 1).

Figure 1: Diagram of the Go/No-go Task. During presentation of a Go stimulus, lever pressing is reinforced on a VI-60 s schedule with one sugar pellet. During presentation of the No-go stimulus, lever pressing is not reinforced.

First, rats (N = 16) were magazine trained for two consecutive days, receiving 20 pellets on a variable interval (VI)-60 s schedule in a 30 min session. Rats were then trained to lever press for food on a continuous reinforcement (CRF) schedule until 100 pellets were earned in a 40 min session for two consecutive days. During CRF training, only one lever was in the chamber and a discriminative stimulus (S_D) was turned on. The assignment of the lever (left or right) and the S_D (house light or lever light) was counterbalanced across rats. The reinforced lever and S_D that were used in CRF training were used in the remaining phases of the experiment. The magazine light signaled food delivery during all phases of the experiment. Following CRF training, rats were trained
on a VI-20 s schedule of reinforcement for two consecutive days. This was followed by Go/No-go testing, with Go and No-go intervals alternating every 2 min across the 40 min session. Lever presses during Go intervals were reinforced on a VI-20s schedule; lever presses during No-go intervals were not reinforced. Go intervals were signaled by the same SD used during CRF training. During Go intervals the lever remained extended and the SD remained illuminated except when the food pellet was delivered to the food magazine. At this point the magazine light was illuminated, the lever retracted and the SD light turned off for 1 s. During the 2-min No-go intervals, responding did not result in reinforcement.

Training continued until the percentage of Go responding reached a group mean of 80% for three consecutive days. Each session length was 40 min; subjects were tested one session per day. Increased motor impulsivity is marked by no change in responding during the Go intervals, and increased responding during the No-go intervals (Fletcher, 1993).

Rats had four days of baseline sessions; the means of the four sessions were used to assign rats to the control or stress group. Rats were equated for lever (left or right), discriminative stimulus (house light or lever light), number of Go interval responses, number of No-go interval responses and percentage of Go responding (measured as # Go interval responses / (# Go interval responses + # No-go interval responses)*100). Following the four baseline sessions, the stress group was tested immediately following one hour of stress on three consecutive sessions; the control group was tested in the absence of stress.
Experiment 3: Delayed Reinforcement. The ability to delay gratification was assessed using a Delayed Reinforcement paradigm, based on procedures described by Evenden and Ryan (1996), Cardinal et al. (2000) and Paine, Dringenberg, and Olmstead (2003) (Figure 2).

Figure 2: Diagram of the Delayed Reinforcement task. During the Delayed Reinforcement task, responses on one lever produce one sugar pellet following a 0 s delay whereas responses on the other lever produce 5 sugar pellets following a 0 – 60 s delay.

Rats (N = 16) were magazine trained for two days, with the magazine light acting as a S_D. Following magazine training, rats were trained on a CRF schedule, with the reinforced lever (left and right) alternating across days. Training continued until rats obtained 100 pellets in 20 min for two consecutive days. Rats were then trained on a simplified version of the Delayed Reinforcement test. Trials begin every 40 s with the illumination of the house light and magazine light. Rats were required to nose poke in the food magazine within 10 s, or the trial ended and the chamber returned to darkness for the remainder of the trial. If a rat did nose poke, the magazine light turned off, and a single lever was presented (trials were presented in pairs with the right and left lever randomly presented once). If the rat then responded on the lever within 10 s, a single
pellet was dispensed in the food magazine, and the magazine light was illuminated until
the rat retrieved the food, or 6 s elapsed. If the rat failed to lever press within 10 s, the
chamber returned to darkness for the duration of the trial and no food was dispensed.
Training continued until rats completed 60 trials in 1 hr for two consecutive days.

Once criterion was reached, rats were tested on the full Delayed Reinforcement paradigm. Trials begin every 100 s with the chamber in darkness and both levers
retracted (i.e., inter-trial state). The trial was signaled by the illumination of the house
light and magazine light. If the rat nose poked within 10 s from the start of the trial, both
levers were presented and the magazine light extinguished. If the rat did not nose poke
within 10 s, a “light” omission was recorded, and the trial continued. Rats were required
to respond on either lever within 10 s or the chamber returned to the inter-trial state for
the duration of the 100s and the trial was recorded as a “lever” omission. After one of the
levers was chosen, both levers retracted and the house light turned off. Choice of the
Immediate lever dispensed one sugar pellet after a 0-s delay; choice of the Delay lever
dispensed five sugar pellets following a delay of 0, 10, 20, 40, or 60 s. Following the
choice of either the Delay or Immediate lever, the magazine turned on, and remained on
until the rat retrieved the food or 6 s elapsed. Following this, the chamber remained in
darkness (i.e., inter-trial state) for the remainder of the 100 s trial. The Immediate and
Delay lever side remained consistent across both trial and session; assignment of
Immediate and Delay lever was counterbalanced (left or right), with the left lever being
the Delay lever for half of the rats, and the right lever being the Delay lever for the other
half.
Each session consisted of five blocks of 12 trials, with the reinforcement delay varying across blocks. The first two trials of each block were forced choices in which only one lever was presented (one trial for each lever, in randomized order). In the remaining 10 trials both levers were presented. For half the rats, delays were presented in ascending order (0 through 60 s) and descending order (60 through 0 s) for the other half of the rats.

In each session, the percent choice of the delay lever (# delay lever responses/total responses), indifference point and the slope were calculated. The indifference point was calculated as $D_{50} = d_i + (d_j - d_i) \times \left[ \frac{(\%B \text{ at } d_i - 50)}{(\%B \text{ at } d_i - \%B \text{ at } d_j)} \right]$, where $B$ is the Delay lever, $d_i$ is the delay when less than 50% of delay responses are made, $d_j$ is the delay when more than 50% delay responses are made. The indifference point represents the time when a rat is responding equally on both the Delay and Immediate levers. The slope of the linear regression of % choice of the large reinforcer against log (delay + 1 s) was also determined (Cardinal, Robbins, & Everitt, 2000). The number of light and lever omissions were also recorded, both of which are measures of attention. The assignment of Immediate and Delay levers, as well as the order of delay presentation (ascending versus descending) were counterbalanced across animals. Each session was 100 min with one session per day for each subject.

Training continued until there was less than 10% variability in the slope for three consecutive sessions. Once rats reached this criterion of stability, they were assigned to either the control or stress group. Groups were counterbalanced to ensure the groups were equal regarding: indifference point values, slope, order of latency of the Delay lever.
(ascending or descending), and Delay lever (left or right). T-tests were conducted to ensure that there are no group differences for indifference point and slope.

Following the four baseline sessions, the stress group was tested following one hour of stress on three consecutive sessions; the control group was tested in the absence of stress. Increases in the indifference point value were used as an index of increased impulsivity.

**Data Analysis**

All analyses were performed using the Statistical Package for the Social Sciences (SPSS). All tests of significance were tested at $\alpha = 0.05$. Violations of sphericity for within-subjects measures were assessed using Mauchly’s test, and degrees of freedom were adjusted with the Huynh-Feldt epsilon.

**Pilot Testing.** To determine if 60 min of restraint stress induces behavioural effects, independent samples $t$-tests were used to compare group differences (stress versus control) in the percentage of time spent on the open arm, percentage of open arm entries, and total number of closed arm entries.

**Experiment 1: Corticosterone reactivity.** Plasma corticosterone concentration levels were determined at five sampling points: 0 min (baseline), 15 min, 60 min, 100 min and 180 min. Using these data, the area under the curve (AUC) was calculated using the formula,

$$AUC_i = \left( \sum_{i=1}^{n-1} \left( \frac{m_i + m_{i+1}}{2} \cdot t_i \right) \right) - \left( m_1 \sum_{i=1}^{n-1} t_i \right)$$
with $m_i$ denoting the single assay values, $t_i$ denoting the time distance between the measurements, and $n$ denoting the total number of measurements. This formula takes into account the time between samples, and the concentration of corticosterone to determine the area that is representative of overall corticosterone release over time (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

AUC data were analyzed using an independent samples $t$-test, with Group (stress or control) as a between-subjects factor. In order to determine the specific differences in corticosterone concentration at the 5 sampling points, corticosterone data were analyzed using two mixed factorial ANOVAs. In the first ANOVA, Time (0 min, 15 min, 40 min, 100 min, and 180 min) was the within-subjects factor, and Group (stress or control) was the between-subjects factor. The dependent variable was the total concentration of corticosterone at each time point. In the second ANOVA, the percentage change from baseline was the dependent variable, with Time (15 min, 40 min, 100 min, 180 min) as the within-subjects factor, and Group (stress or control) as the between-subjects factor. To examine simple main effects in the mixed model ANOVA, post-hoc independent samples $t$-tests were used, setting alpha to .05 divided by the total number of $t$-tests, to correct for family-wise error (Howell, 2003).

Experiment 2: Go/No-go. Independent samples $t$-tests were used to ensure that groups were equated at baseline with respect to all dependent measures of the Go/No-go test: percentage Go interval responding, number of Go interval responses, number of No-go interval responses, total number of rewards, and latency to respond during the Go intervals. Baseline data were calculated as the mean of the three consecutive days of pre-
stress testing; test data were calculated as the mean of the three consecutive days of stress testing.

Data from test sessions (percentage Go responding, total number of Go interval responses, total number of No-go interval responses, latency to respond on the go lever and total rewards received in a session) were analyzed using a 2-way mixed-model factorial ANOVA, with Time (baseline or test) as a within subjects variable, and Group (stress or control) as a between subjects variable.

Percentage of Go responding was also assessed during the first 5 intervals of Go/No-go testing to determine if responding differed during the first half of Go/No-go testing. A 2-way mixed-model factorial ANOVA was used, with Time (baseline or test) as a within subjects variable, and Group (stress or control) as a between subjects variable.

Experiment 3: Delayed Reinforcement. To ensure no group differences at baseline, percent choice of the delay lever during baseline sessions was calculated using a 3-way mixed-model factorial ANOVA, with Group (stress or control) and Order (ascending or descending) as between-subjects factors, and Delay (0, 10, 20, 40, and 60 s) as a within-subjects factor. Indifference points were analyzed using a 2-way ANOVA (Group × Order). Baseline data were calculated as the mean of the last three consecutive pre-stress sessions; test data were calculated as the mean of the three consecutive days of stress testing.

Data from test sessions of the Delayed Reinforcement test (indifference point, latency to respond on the delay lever, latency to respond on the immediate lever, number of delay lever responses and number of immediate lever responses) were analyzed using a 2-way mixed-model factorial ANOVA with Time (baseline and test) as a within-
subjects variable and Group (stress or control) as a between-subjects variable. Group
differences in the number of omissions was assessed using the non-parametric Mann-
Whitney U measure, whereby values for each group are ranked by number of omissions,
and analyses are performed on the ranked values.

Percent choice of the delay lever was analyzed using a 2-way mixed factorial
ANOVA, with Delay (0-60 s) as the within subjects variable, and Group (stress or
control) as the between subjects factor.
Chapter 3
Results

Pilot Testing

Figure 1 shows the effect of the stress manipulation on the EPM test. The stress group spent significantly less time on the open arms, $t(5.936) = 3.52, p < .05$. There were no group differences in the percentage of open arm entries, $t(10) = 1.30, p = .22$, or in the total number of closed arm entries, $t(10) = 1.70, p < .12$. 
Figure 3: Elevated plus maze arm activity.
Mean (± SEM) percentage of time spent on the open arms (top), percentage of open arm entries (middle), and total number of closed arm entries (bottom) over a 5 min test in the elevated plus maze (EPM) for control ($n = 6$) and stress ($n = 6$) groups. * $p < .05$.

**Experiment 1: Corticosterone reactivity**

Data from two rats were discarded from the corticosterone reactivity analysis; a baseline sample could not be attained from one animal in an appropriate length of time and
samples from a second rat were discarded because the baseline sample was too small to analyze. Data from the remaining 18 rats were used in the following analyses.

Of the 95 samples taken, 23 samples could not be analyzed due to procedural issues (e.g., the blood samples were too small for analysis or the duration of blood sampling exceeded 3 min). Of the remaining 77 samples, 62 were analyzed in duplicate, and the remaining 15 were analyzed in singlet. To measure the reliability of the assay values, a coefficient of variation (CV) was calculated for duplicate samples. A CV value of less than 10% is considered good reliability (Schultheiss & Stanton, 2009). Of the duplicate samples, 51 samples had a CV of less than 5%, and only three samples had a CV above 10%. Due to these superior CV values, there is confidence in the reliability of the singlet corticosterone values. More specifically, assay variability was calculated from a standard at 512.23 ng/ml, yielding an intra-assay CV of 7.7% and an inter-assay CV of 9.7%.

Baseline. An independent samples t-test confirmed that there were no baseline differences in plasma corticosterone concentrations between groups \( t(15) = .81, p > .05 \) (control \( M = 513.76, SD = 170.65; \) stress \( M = 594.12, SD = 238.21 \)).

Test. Analysis of absolute plasma corticosterone concentrations revealed a significant Time \( \times \) Condition (Figure 2) interaction, \( F(4, 60) = 11.57, p < .01 \), and significant main effects of Time, \( F(4, 60) = 14.58, p < .01 \), and Condition, \( F(1, 15) = 6.41, p < .05 \). Post-hoc independent samples t-tests revealed that the stress group had a significantly higher percentage change from baseline than the control group at 15 min and 60 min, \( p < .05 \).
Figure 4: Absolute plasma corticosterone concentrations. Mean (± SEM) absolute plasma corticosterone concentrations at baseline (0 min), and four sampling points following baseline (15 min, 60 min, 100 min, and 180 min) for the stress (n = 7) and control (n = 8) groups in Experiment 1. * p < .05. The black vertical line represents the duration of the restraint stress.

Analysis of percentage change from baseline demonstrated a significant Time × Condition interaction, $F(3, 39) = 6.57, p < .01$, and a significant main effect of Time, $F(3, 39) = 7.79, p < .01$, but not Condition, $F(1, 13) = 3.25, p = .32$. Post-hoc independent samples $t$-tests revealed that the stress group had a significantly higher percentage change from baseline than the control group at 15 min and 60 min only, $p < .05$. 
Figure 5: Percentage change in plasma corticosterone concentration. Mean (± SEM) percentage change in plasma corticosterone concentration from baseline values at the four sampling points following baseline (15 min, 60 min, 100 min, and 180 min) for the stress (n = 7) and control (n = 8) groups in Experiment 1. * p < .05.

The AUC measure verified that the stress group experienced a greater overall corticosterone release than the control group, t (13) = 2.33, p < .05 (Figure 4).

Figure 6: Corticosterone responses to stress measured as area under the curve. Mean (± SEM) of area under the curve data for stress (n = 7) and control (n = 8) groups in Experiment 1. AUC is a measure of overall corticosterone reactivity, which takes into account the absolute plasma corticosterone concentration and the time between samples (see data analysis for details). * p < .05.
Experiment 2: Go/No-go

Baseline performance. Groups did not differ at baseline on any of the Go/No-go dependent measures (Table 1).

Table 1: *Independent t-tests for Baseline Measures of the Go/No-go Test*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>M</th>
<th>SD</th>
<th>df</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Go Responding</td>
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<td>13</td>
<td>.47</td>
<td>.65</td>
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<tr>
<td></td>
<td>Stress</td>
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<tr>
<td></td>
<td>Control</td>
<td>82.14</td>
<td>10.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Number of Go interval</td>
<td></td>
<td>13</td>
<td>.60</td>
<td>.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responses</td>
<td></td>
<td>13</td>
<td>.13</td>
<td>.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stress</td>
<td>1491.78</td>
<td>362.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1368.89</td>
<td>427.49</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total Number of No-go interval</td>
<td></td>
<td>13</td>
<td>.50</td>
<td>.63</td>
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<tr>
<td>Responses</td>
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<td>.48</td>
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<td>Stress</td>
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<td></td>
<td>Control</td>
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<td>4.51</td>
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<td></td>
</tr>
<tr>
<td>Total Number of Rewards</td>
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<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stress</td>
<td>43.00</td>
<td>3.28</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>44.00</td>
<td>4.51</td>
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</tr>
</tbody>
</table>

Test. There were no significant main effects or interactions for percentage of Go interval responding or total number of No-go interval responses (Table 2, Figures 5, 6).
Figure 7: Percentage of Go interval responding during Go/No-go testing. Mean (± SEM) percentage of Go interval responding at baseline and test for the stress ($n = 8$) and control ($n = 7$) groups during Go/No-go testing.
A significant main effect of Time and a significant Time × Group interaction was observed for the number of Go interval responses, whereby the stress group made significantly fewer Go interval responses than the control group during the testing phase (Table 2, Figure 6).

Figure 8: Number of Go and No-go interval responses during Go/No-go testing. Mean (± SEM) Number of Go interval responses (top) and No-go interval responses (bottom) at baseline and test for control (n = 7) and stress (n = 8) groups during Go/No-go testing. * p < .05.

For the total number of rewards, there was a main effect of both Group and Time and a significant Time × Group interaction, whereby the stress group received significantly fewer rewards at test than the control group (Table 2, Figure 7).
Figure 9: Number of rewards received during Go/No-go testing. Mean (± SEM) number of rewards received during the Go intervals at baseline and test for the stress ($n = 8$) and control ($n = 7$) groups during Go/No-go testing. * $p < .05$.

As shown in Figure 8, the stress group was significantly slower than the control group in responding on the Go interval lever at test, but not at baseline, manifested as a significant session $\times$ group interaction. The main effects, Time and Group were not significant (Table 2).

Figure 10: Latency to respond (s) during the Go intervals of Go/No-go testing. Mean (± SEM) latency to respond (in seconds), during the Go intervals at baseline and test, for the stress ($n = 8$) and control ($n = 7$) groups during Go/No-go testing. * $p < .05$. 

26
Table 2: Mixed Model ANOVA for Dependent Measures of the Go/No-go Test

<table>
<thead>
<tr>
<th>Measure</th>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>.22</td>
<td>.65</td>
</tr>
<tr>
<td></td>
<td>Time</td>
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<td>.81</td>
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<td>.00</td>
<td>.95</td>
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<td>Time</td>
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<td></td>
<td>Time × Condition</td>
<td>1,13</td>
<td>15.28</td>
<td>.01*</td>
</tr>
<tr>
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<td>.26</td>
<td>.62</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>1,13</td>
<td>1.68</td>
<td>.22</td>
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<td></td>
<td>Time × Condition</td>
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<td>7.40</td>
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<td></td>
<td>Time</td>
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<td></td>
<td>Time × Condition</td>
<td>1,13</td>
<td>6.68</td>
<td>.05*</td>
</tr>
</tbody>
</table>

* p < .05

In order to examine whether the effects of stress on motor impulsivity were masked by the long testing period, the stress and control groups were compared during the first five intervals of the Go/No-go testing. This analysis revealed no significant main effects of Time, F (1, 13) = .02, p < .05 or Group, F (1, 13) = .24, p < .05, and no
significant Time × Group interaction for percentage of Go responding, $F(1, 13) = .001, p < .05$ (Figure 9).

Figure 11: Percentage of Go responding during the first 5 intervals of Go/No-go testing. Mean (± SEM) percentage of Go responding at baseline and test for the stress ($n = 8$) and control ($n = 7$) groups during the first 5 intervals of Go/No-go testing.

**Experiment 3: Delayed Reinforcement**

Baseline performance. There were no significant main effects of Group or Order and no significant interactions for percentage choice of the delay lever at baseline (Table 3). As expected, there was a significant main effect of Time, whereby percentage choice of the delayed lever decreased as the delay increased (Table 3).

<table>
<thead>
<tr>
<th>Table 3: Mixed Model ANOVA for Percent Choice of the Delay Lever at Baseline in the Delayed Reinforcement Test</th>
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<tbody>
<tr>
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<td>Time</td>
</tr>
<tr>
<td>Time × Condition</td>
</tr>
<tr>
<td>Time × Order</td>
</tr>
<tr>
<td>Time × Condition × Order</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Condition</td>
</tr>
<tr>
<td>Condition × Order</td>
</tr>
</tbody>
</table>

* * *

Groups did not differ in indifference point values at baseline, $F (1, 10) = 2.21$, $p < .05$, and there was no significant effect of Order, $F (1, 10) = .60$, $p < .05$ or significant Group × Order interaction, $F (1, 10) = .03$, $p < .05$.

Test. Table 4 presents statistics for indifference point, latency to respond on the delay lever, latency to respond on the immediate lever, number of delay lever responses and number of immediate lever responses. There were no significant main effects or interactions in indifference point values (Figure 10), latency to respond on the immediate lever (Figure 11), number of delay or immediate lever responses (Figure 12). For latency to respond on the delayed lever, the Condition main effect was not significant, but there was a significant main effect of Time, and a Time × Condition interaction, whereby the stress rats took significantly longer to respond on the delay lever at test than the control group (Figure 11).
Figure 12: Indifference points from Delayed Reinforcement testing. Mean (± SEM) indifference point at baseline and test for the stress ($n = 8$) and control ($n = 6$) groups during Delayed Reinforcement testing.

Figure 13: Latency (s) to the first response in each block on the immediate and delay lever during Delayed Reinforcement testing. Mean (± SEM) latency (s) to the first response in each block on the immediate lever (top) and delay lever (bottom) at baseline and test for the stress ($n = 8$) and control ($n = 6$) groups during Delayed Reinforcement testing. * $p < .05$. 
Figure 14: Number of responses on the immediate and delay levers during Delayed Reinforcement testing. Mean (± SEM) number of responses on the immediate (top) and delay (bottom) levers at baseline and test for the stress ($n = 8$) and control ($n = 6$) groups during Delayed Reinforcement testing.

Table 4: *Mixed Model ANOVA for Dependent Measures of the Delayed Reinforcement Test*

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<td></td>
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<td>.36</td>
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<td>Time × Condition</td>
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<tr>
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</tr>
<tr>
<td><strong>Time × Condition</strong></td>
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<td><strong>p</strong></td>
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<table>
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</tr>
<tr>
<td>Time × Condition</td>
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<tr>
<td><strong>Condition</strong></td>
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<tr>
<td><strong>Time</strong></td>
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<tr>
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</tr>
<tr>
<td>Time × Condition</td>
<td>1,12</td>
</tr>
<tr>
<td><strong>Condition</strong></td>
<td><strong>1,12</strong></td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td><strong>1,12</strong></td>
</tr>
<tr>
<td><strong>Time × Condition</strong></td>
<td><strong>1,12</strong></td>
</tr>
<tr>
<td><strong>F</strong></td>
<td><strong>2.21</strong></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td><strong>.16</strong></td>
</tr>
<tr>
<td><strong>F</strong></td>
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<tr>
<td><strong>p</strong></td>
<td><strong>.38</strong></td>
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<tr>
<td><strong>F</strong></td>
<td><strong>.62</strong></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td><strong>.45</strong></td>
</tr>
</tbody>
</table>

* *p < .05

There were no baseline differences in the number of omissions between the stress and control groups, $z = .95, p = .34$; however, the stress group made a significantly greater number of omissions than the control group at test, $z = 2.84, p < .05$ (Figure 13). At baseline, the stress group had a mean rank of 7.93, and the control group had a mean rank of 5.92. At test, the stress group had an average rank of 10.25 omissions, and the control group had a mean rank of 3.83 omissions.
ANOVA revealed a significant main effect of Time for the percent choice of delay lever, whereby increased delays decreased this value, $F(4, 48) = 30.76, p < .05$ (Figure 14). The Time × Condition interaction was not statistically significant, $F(4, 40) = 2.99, p < .05$, nor was the main effect of Condition, $F(1, 12) = .25, p < .05$.

Figure 16: Percentage choice of the delay lever at each delay (s) during Delayed Reinforcement testing. Mean (± SEM) percentage choice of the delay lever at each delay (in seconds), at test phase for the stress ($n = 8$) and control ($n = 6$) groups during Delayed Reinforcement testing.
Chapter 4
Discussion

The current study investigated the role of 1 hr of acute restraint stress on two behavioural measures of impulsivity in rats. Results from pilot data and Experiment 1 show that 1 hr of restraint stress produces behavioural indices of anxiety and is physiologically stressful, as measured by increased plasma corticosterone levels 15 min into the stressor, and immediately following the stressor. Regardless, there were no changes in responding following stress on the two principal measures of impulsivity. Percentage of Go interval responding did not change in the Go/No-go test, nor did indifference points change in the Delayed Reinforcement test. Together, these results suggest that acute stress does not affect, or interact with, either motor or cognitive impulsivity, as measured in these tasks.

Although stress did not affect measures of impulsivity in either the Go/No-go or Delayed Reinforcement tests, the manipulation did alter other indices of responding. In the Go/No-go test, stressed rats made fewer Go interval responses, but there were no changes in No-go responses. This difference is likely due to the already low No-go interval responding during baseline sessions, which did not allow for further decreases in responding during testing. Furthermore, in the Go/No-go test, stressed rats received fewer rewards, which is not surprising, given that they made fewer Go interval responses. Finally, stress increased the latency to respond during the Go intervals. Similarly, during the Delayed Reinforcement test, stress increased the latency to respond on the delay lever, and the number of omissions across the sessions. Based on these changes, the inability of stress to modulate cognitive or motor impulsivity is unlikely to be due to an ineffective stressor.
Increased latency to respond on both tasks and more frequent omissions on the delayed reinforcement task may suggest that stress impairs attentional processes, independent of impulsivity. Both the Go/No-go and Delayed Reinforcement task require prolonged attention to the cues (e.g., discriminative stimulus). This is supported by evidence that human participants with high levels of cortisol have decreased performance in a task requiring prolonged attention (Bohnen, Houx, Nicolson, & Jolles, 1990). However, other evidence suggests that stress may enhance rather than impair attentional processing. Intracerebroventricular injections of CRF (an endogenous peptide secreted in response to stress) in rats increases, rather than decreases, attention in the 5-choice serial reaction time task (5-CSRTT; Ohmura, et al., 2009). How these data fit with the current study is unclear; the role of endogenous CRF in attentional processes measured on the 5-CSRTT is unknown, and therefore, results are not directly comparable to the present study. Because the 5-CSRTT provides independent measures of impulsivity and attention, it would be an appropriate measure to dissociate the effects of impulsivity and attention following a 1 hr restraint in future studies.

It is unlikely that increased latencies and omissions reflect a change in motivational state because there are no differences in responding on the delayed lever in the Delayed Reinforcement task at the 0 s delay. More specifically, rats received an equal number of reinforcers from the delay and immediate levers at the 0 s delay. Also, animals consumed all of the delivered sucrose pellets suggesting that they did not suffer any primary motivational deficit.

The negative findings in this study could also reflect the fact that neither the Go/No-go test nor the Delayed Reinforcement test are appropriate paradigms to study the
effects of stress on impulsivity. The Go/No-go test measures the ability to withhold a response. Stress may not affect this form of impulsive behaviour, but may affect the ability to stop an already initiated response, a process that is measured in the stop-task. Also, further increases in percentage Go responding are unlikely because of already low levels of No-go interval responding and high levels of Go interval responding. Thus, the test is not sensitive enough to measure decreases in impulsivity, which would be manifested as an increase in the percentage of Go interval responding. As a result, the Go/No-test may not be able to test the alternative hypothesis that stress decreases impulsivity. A task such as the Differential-reinforcement of Low Rate (DRL) schedule, which reinforces the first response following the previous response by a minimum time period, allows for changes in responding in both directions to be observed.

Unlike motor impulsivity, there is one ‘gold standard’ test of cognitive impulsivity. The Delayed Reinforcement paradigm is used in both humans and animal research to measure this process. The dependent measure in the task, indifference points, is sensitive to both increases and decreases in impulsivity, so it is unlikely that the null findings are due to ceiling effects in this task.

Other explanations for the inability of stress to impact impulsivity levels may be related to the stressor itself, and its role in activating the HPA system. Following a stressor, corticotropin-releasing factor (CRH), and the activation of CRH receptor 1 (CRH₁) occurs rapidly. CRH then subsequently activates the HPA system including corticosterone, which is also very fast (Akil & Morano, 1996). Stress-induced changes in corticosterone concentrations may have returned to sub-threshold plasma corticosterone
concentration levels too quickly for changes in motor and cognitive impulsivity to be observed.

One alternative is that stress induces physiological changes that take some time to develop, similar to the incubation effect that is described for drug administration, most commonly observed during withdrawal (Cullinan, Herman, Battaglia, Akil & Watson, 1995; Lu, Grimm, Dempsey, & Shaham, 2004). Similarly, animals show increased anxiety following acute restraint stress 24 hrs later (Netto, Silveira, Coimbra, Joca, & Guimarães, 2002; Resstel, et al., 2009). It may be this period that is also important for the effect of stress on impulsivity. Although the hypothesis is plausible, it is unlikely that this affected the results of the present study; stress rats were restrained on three consecutive days and no differences were found using the two measures of impulsivity (assessed as the % Go responding or the indifference point).

In a similar vein, while both acute and chronic stress increase one’s risk for initiating and maintaining drug use, as well as for relapse (Koob, 2008; Wilsnack et al., 1997), chronic stress may be more effective than acute stress in altering impulsivity. There are several common brain areas implicated in both the effects of stress and impulsivity, namely the orbitofrontal cortex, medial prefrontal cortex and basolateral amygdala (Koob, 2008; Olmstead, 2006). Chronic restraint stress induces retraction and debranching of apical dendrites in the orbitofrontal cortex and medial prefrontal cortex (Liston, et al., 2006; Radley, et al., 2006), effects not observed following acute stress. These brain adaptations following chronic stress may cause changes in impulsivity.

This study examined how an acute manipulation (i.e., a stressor) alters impulsivity, thereby providing a measure of state impulsivity. In contrast, trait
impulsivity describes a stable personality trait that is invariant across situations (de Wit, 2009). Although it was not tested specifically, changes in state impulsivity may depend on baseline levels of trait impulsivity. For example, individuals that are already highly impulsive may be more or less likely to show alterations in impulsivity following a stressor. This possibility could be tested in studies that used larger sample sizes and provided a baseline measure of impulsivity that would allow for division of high and low impulsive groups.

In relation to drug abuse, trait impulsivity relates to the pre-drug brain that increases one’s risk for drug abuse; state impulsivity relates to the changes to the brain following drug use (for a review see Perry & Carroll, 2008). Animals that are identified as high impulsive based on a motor impulsivity task (trait impulsivity) are more likely to self-administer cocaine at high rates than are animals identified as low impulsive (Dalley, et al., 2007). Similarly, increased cognitive impulsivity predicts drug use in both animals and humans (Perry, Larson, German, Madden, & Carroll, 2005).

Tests for state impulsivity commonly occur following either acute or chronic drug administration. Such findings indicate both humans and animals display impaired behavioural inhibition (Fillmore & Rush, 2002) and increased cognitive impulsivity (Bickel & Marsch, 2001; Kirby & Petry, 2004; Simon, Mendez, & Setlow, 2007) following chronic drug administration. In the context of drug abuse, it is possible that the same changes that occur to brain circuitry following drug use may be important for any interactions between impulsivity and stress.

It is clear that both stress and impulsivity have important roles in all phases of addiction, including the initiation and maintenance of drug addiction, as well as relapse to
drug use following abstinence (Goeders, 1997; Lu, Shepard, Hall, & Shaham, 2003; Olmstead, 2006). The results of the present study show that stress does not interact with impulsivity, although alternate explanations for these null effects must be considered. While stress and impulsivity recruit many of the same neural systems, the present results suggest different mechanisms of action, and independent influences of acute stress and impulsivity in their roles of drug abuse.
References


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