BEHAVIORAL ENDOPHENOTYPES OF CHRONIC UNPREDICTABLE STRESS

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BEHAVIORAL ENDOPHENOTYPES OF CHRONIC UNPREDICTABLE STRESS

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Dedication

To my friends, who've managed to retain their offensive and odd sense of humor despite what life throws at them.

Abstract

Stress is the response to a perceived or real threat. Acute incidences of stress can be beneficial. However, repeated stress can cause maladaptive changes in brain and behavior leading to neuropsychiatric illnesses. Among publications on chronic stress, only recently has its effects on decision-making been identified. I have designed a competitive choice task (CCT) to measure changes in decision-making. Decision-making behaviors in this task are governed by the dorsal lateral striatum, ventral striatum and medial prefrontal cortex. These behaviors that these structures mediate are lose-shift, win-stay, and extraneous feeder sampling, respectively. I find that chronic unpredictable stress increases win-stay behavior strategies, decreases extraneous feeder sampling and increases speed but does not affect lose-shift strategies. Chronic stress, therefore, increases activity within the ventral striatum and decreases activity within the medial prefrontal cortex.

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Thank you to my family and my significant other for their support and for handling my crying fits.

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List of abbreviations

HPA	Hypothalamic-pituitary-adrenal axis
PVN	Paraventricular nucleus
CRH	Corticotropin-releasing hormone
ACTH	Adrenocorticotropic releasing hormone
MR	Mineralocorticoid receptors
GR	Glucocorticoid receptors
PFC	Prefrontal cortex
BLA	Basolateral amygdala
OFC	Orbital-frontal cortex
DLS	Dorsal lateral striatum
VS	Ventral striatum
DMS	Dorsal medial striatum
CCT	Completive choice task
EFS	Extraneous feeder sampling
ITI	Inter trial interval
ACC	Anterior cingulate cortex
FST	Forced swim test
OFT	Open field test
ANOVA	Analysis of variance

1. Introduction

1.1 Prevalence of stress disorders

The occurrence of mental health problems have been the subject of intrigue and speculation (Arnetz & Ekman, 2006). Mood and anxiety disorders are the most commonly diagnosed illnesses in the DSM-V (Arnetz & Ekman, 2006). Stress disorders are so prevalent in Canada that as many as 1 in 4 people are affected at least once in their lifetime. The economic burden due to treatment, loss in productivity, and compensation is estimated to be $23 billion annually (Arnetz & Ekman, 2006; Radley, Morilak, Viau, & Campeau, 2015). Although progress in treatments have advanced, it is difficult to ignore patients who do not respond to treatment (Frodl & O'Keane, 2013). The goal of this thesis is to identify the causes and manifestations of mood disorders, which, in animal studies, are induced by the administration of chronic and repeated stressful stimuli. Such studies in humans are limited by ethical constraints and so motivates the use of nonhuman animal models. Although animal models may not accurately represent the full extent of mental health disorders, they show similar underlying behavioral endophenotypes (McEwen et al., 2015; Sapolsky, 2004).

2. Background

2.1 The stress response

Selye (2013) in his formative theory, the general adaptive syndrome theory, argues that an organ displays three general responses to stress, arousal, resistance and collapse. It would therefore be expected that the brain would respond similarly. Stress is the body's response to a perceived or real threat. In response to stress, the brain releases

neurotransmitters and hormones to cope with these immediate threats while attempting to remove the physical effects of stress (Arnetz & Ekman, 2006). Features of the stress response are that in arousal, it activates multiple neurophysiological pathways. These include limbic-forebrain circuits, hypothalamic-pituitary-adrenal axis (HPA), and the autonomic sympathetic response (Arnetz & Ekman, 2006; McEwan et al., 2015; Radley et al., 2015). In resistance there is a hypervigilant fight-or-flight reaction as cortisol, epinephrine, norepinephrine and other monoamines flood the brain (Radley et al., 2015). As will be described below, collapse might be featured in changes in various brain areas and pathways.

The HPA axis involves the paraventricular nucleus (PVN) in the hypothalamus, which releases corticotropin-releasing hormone (CRH) and targets the anterior pituitary gland to release adrenocorticotropic releasing hormone (ACTH) into the bloodstream (Frodl & O'Keane, 2013). ACTH targets the adrenal glands to release further glucocorticoids such as cortisol to target receptors in the hippocampus, hypothalamus, amygdala, prefrontal cortex (PFC), and other extrahypothalamic regions (Frodl & O'Keane, 2013; McEwan et al., 2015). Activation of these regions also allows for negative feedback regulation at the site of CRH and ACTH release (Frodl & O'Keane, 2013). This results in termination of the HPA axis (Frodl & O'Keane, 2013).

Cortisol is the active stress hormone in most primates, whereas corticosterone is the primary stress hormone in rodents. These glucocorticoids target either mineralocorticoid receptors (MRs) or glucocorticoid receptors (GRs) (Arnetz & Ekman, 2006; McEwan et al., 2015). MRs have a higher affinity for glucocorticoids and are usually saturated at basal levels whereas GRs have a lower affinity and bind at higher glucocorticoid levels (Arnetz

& Ekman, 2006). Once the HPA axis is activated, it is the GRs upon activation, will inhibit the further release of glucocorticoids (Arnetz & Ekman, 2006).

2.2 When the stress response becomes maladaptive

Although acute incidences of stress can be beneficial in responding to environmental stimuli, repeated stressful events result in brain and body modifications. The wear and tear on the brain and the body is referred to as allostatic load (McEwan et al., 2015). Chronic stress or "allostatic overload", leads to altered glucocorticoid and monoamine production, decreased neurogenesis and disrupted stress feedback loops (McEwen et al., 2015).

Brain structures that might be impacted by chronic stress include the amygdala, hippocampus, the thalamus, orbital frontal cortex (OFC), and the prefrontal cortex (PFC) (Bennur et al.,2007; McEwen et al., 2015). Repeated stress-driven activation of the HPA axis and neuroplastic changes within these structures is associated with several deficiencies including: impairment in memory, impulse, hypervigilance, disrupted circadian rhythms, cognitive ridgity, depression, anxiety, and post-traumatic stress disorder (PTSD) (Arnetz & Ekman, 2006; Bennur et al.,2007; McEwen et al., 2015; Sapolsky, Krey, & McEwan, 2002).

In normal subjects, the HPA axis is thought to be regulated by inhibitory regions such as the hippocampus (McEwan et al., 2015). Activation of this area provides negative feedback to the paraventricular nucleus resulting in termination of the HPA axis (Arnetz & Ekman, 2006; McEwan et al., 2015). With chronic stress, GRs in the hippocampus show decreased sensitivity to circulating levels of glucocorticoids (Arnetz & Ekman, 2006;

McEwan et al., 2015). The hippocampus is responsible for turning off GR activation. Therefore, changes in the GRs lead to loss of negative feedback (Sapolsky et al., 2002). In addition to the hippocampus, the medial PFC is also abundant in GRs and subjected to the same glucocorticoid-induced changes (Cerqueira, Mailiet, Almeida, Jay, & Sausa 2007). Since the hippocampus and the PFC are major inhibitory sites for the release of ACTH, decreased activity, due to GR modifications, prolongs the stress response (Cerqueira et al., 2007, 2005; Sapolsky et al., 2002).

When the feedback loop becomes disrupted by stress, each new stressor has a prolonged effect that can manifest as a behavioral disorder (Hill & McEwen, 2010; McEwen et al., 2015). Although this stress circuit is well understood, its interruption can have many other negative consequences in the brain. One way to investigate these potential changes is through the development of animal models.

2.3 Animal models of stress

Animal models of stress allow for the careful manipulation of experimental stress protocols to measure changes in brain and behavior. Influences on stress severity include the age of stress onset, duration, and administration schedule (Lupien, McEwan, Gunnar, & Heim, 2009). Paradigms are typically split into two types, early life stress, including pre or postnatal, and adult or adolescent stress (Lupien et al., 2009). The following sections will describe commonly used stress protocols including: restraint stress, elevated platform stress, the chronic unpredictable/mild stress paradigm, and chronic social defeat stress.

2.3.1 Chronic restraint stress

Chronic restraint stress involves placing the animal in a cylinder to restrict movement. Time in the cylinder may range from 15 min-6 hours. The rationale of the cylinder stress is to simulate a situation where the animal is caught by prey and cannot escape. Fox odor is often used in combination with this stressor, however, restraint alone is a sufficient stressor (Conrad, Magarinos, LeDoux, & McEwan et al., 1999; McLaughlin, Gomez, Baran, & Conrad, 2007; Padival, Blume, Rosenkranz, 2013). To induce chronic stress, the stress is given repeatedly for two weeks to 21 days and can last for 28 days or longer (Padival et al., 2013).

The result on brain and behavioral changes are described by somewhat mixed results in the literature. In a few studies, repeated restraint stress decreased locomotor activity in the open field test, increased freezing during contextual fear conditioning and induced spatial memory impairments during Y-maze testing (Conrad et al., 1999; McLaughlin et al., 2007). On the contrary, another study found that chronic restraint stress increased locomotor activity in the open field test (Strekalova, Spanagel, Dolgov, & Bartsch, 2005). The mixed results might be due to variable testing conditions and durations of stress. Nevertheless, this stress test is a highly reliable and widely used stress model (Strekalova et al., 2005).

2.3.2 Elevated platform stress

Elevated platform stress consists of placing the rat on a 21 x 21 cm clear plexiglass platform 1 m high, twice a day, for a minimum of 30 minutes for 2-5 weeks (Mychasiuk et al., 2016). Due to its relative safety, this stressor can also easily be used in prenatal stress paradigms (Mychasiuk, Gibb, & Kolb, 2011). This stressor also causes long-lasting

changes in epigenetic expression and behavior of paternally stressed offspring, deeming this a robust and reliable stressor (Mychasiuk et al., 2011).

2.3.3 Chronic mild/unpredictable stress

A recently popular stress model is proposed to cause depressed and learned helplessness in rats is the chronic mild stress paradigm developed by R.J. Katz (Willner, 2017). This model exposes animals to consistent, yet unpredictable, mild stressors and causes changes in brain and behavior (Willner, 2017). Mild stressors include inverting the home cage, exposing the rat to 12+ hours of daylight, soaking the animals home cage in water, exposure to loud noises and social isolation for 5-9 weeks. This treatment causes despair and anxiety like behaviors including anhedonia, weight loss, increased anxiety and cognitive impairments in rodents (Willner, 2017). It is, however, debatable as to what stressors are deemed mild compared to severe and therefore, many of the CMS models may show considerable deviation from published literature if labor or equipment is limited (Willner, 1997, 2017)

2.3.4 Chronic social defeat stress

Social defeat paradigms are argued to be the most accurate in mimicking the chronic stress of humans because rat, like humans, are highly social beings. According to Sapolsky, social dynamics and pressures among groups of the same species are the reason humans and primates are susceptible to disorders associated with chronic stress (Sapolsky, 2004). Studies that use this model (social defeat) mimic situations of social subordination using

resident intruder paradigms to induce cognitive and behavioral alterations (Rygula et al., 2005).

The procedure consists of placing an adult rat (intruder) inside a cage with a larger and more aggressive resident and results in both animals fighting (Rygula et al., 2005). The intruder loses the encounter and the experimenter separates the animals when the intruder displays submissive behavior (Rygula et al., 2005). Similar to other stress models, chronic social defeat stress reduces sucrose preference, social avoidance and increases immobility during the forced swim test (Patel, Anilkumar, Chattarji, & Buwalda, 2018; Rygula et al., 2005).

2.4 Behavior

With the brain primed and sensitive to perceived stressful events, it is no surprise that chronic stress increases anxiety and despair like behavior. Tests used to assess this in rodents include the forced swim test (FST), open field test (OFT) and the elevated plus maze. Within these tests, chronically stressed rodents may exhibit higher levels of immobility (despair), freezing and spend more time within the closed arms of the maze (anxiety) (Pellow, Chopin, File, & Briley, 1985; Sáenz, Villagra, & Trías, 2006). Rodents are often also tested on a sucrose preference test and stressed animals may show decreased preference which, is thought to represent anhedonia (Willner et al., 1987).

Among published works on the effects of stress on anhedonia, despair, anxiety and memory, a phenotype often overlooked is its effects on decision-making and reward processing (Cerqueira et al., 2007; Dias-Ferreira et al., 2009; Koehl et al., 1999; Seckl,

2007). In the following section, I will elaborate on how chronic stress might influence decision-making patterns.

2.5 Decision-making

Animals learn from reinforcement by a neural mechanism involving dopamine (Schultz, 1998). Dopaminergic neurons in the midbrain appear to encode a reward prediction error signal as the neurons increase their firing rate when an unexpected reward is presented and decrease firing after omission or a smaller than expected reward is presented (Schultz, 1998). This error signal is the basis for how humans and animals can use trial-and-error learning to make beneficial decisions in novel environments or tasks (Montague et al.,1996; Pessiglione et al., 2006).

Dopamine projections densely innervate the striatum, a structure thought to be involved in reinforcement-based learning (Frank, Scheres, & Sherman, 2007; O'Doherty, Buchanan, Seymour, & Dolan, 2006). The rodent striatum is theorized to be divided into ventral, dorsomedial, and dorsolateral sub-regions, which may be homologous to the nucleus accumbens, caudate, and putamen in primates (Balleine & O'doherty, 2010). Its suggested that these sub-regions are components of parallel circuits between the cortex, basal ganglia, and thalamus (Alexander, DeLong, & Strick, 1986; Haber, 2003; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). These circuits appear to have distinct information processing capabilities and can interact to control decision-making. For example, instrumental conditioning paradigms suggest that the dorsomedial striatum (DMS) encodes action-association outcomes (Daw, Niv, & Dayan, 2005; Yin, Ostlund, Knowlton, & Balleine, 2005). In contrast, the dorsolateral striatum (DLS) encodes

stimulus-response associations that are built up over repetition (Featherstone & McDonald, 2004; Jog, Kubota, Connolly, Hillegaart, & Graybiel, 1999; Packard & McGaugh, 1996) and are insensitive to devaluation (Gruber & Thapa, 2016). These responses are conceptualized as habits that are engaged reflexively (Jog et al., 1999).

For my thesis, I used a behavioral task to measure differential decision-making patterns in rats. In this competitive choice task (CCT), rats are trained to compete against a computer algorithm to maximize reward (sucrose water) delivered in two adjacent wells after initiating a nose-poke at a central odor port (figure 3). This behavior paradigm can quantify decision-making tactics that rely on regions of the striatum and can be manipulated through drugs of abuse and experience (Kolb, Gorny, Li, Samaha, & Robinson, 2003; Wong et al., 2017). With continuous training, rats subjected to this CCT tend to repeat responses that were rewarded immediately preceding trials (win-stay) and switch choices if their responses were not rewarded (lose-shift). With lesions studies and pharmacology, it is found that the DLS influences lose-shift responding whereas the ventral VS is correlated with win-stay responding (Gruber & Thapa, 2016; Skelin et al., 2014). The medial PFC may also influence decision-making strategies and is thought to influence a behavior called extraneous feeder sampling (EFS) (Gruber, Thapa, & Randolph, 2017). Although this behavior is never reinforced and remains persistent, EFS is the phenomenon where, instead of initiating another trial, rats will investigate the other reward well. Recent data suggests that this behavior resembles novelty seeking or exploration (Gruber et al., 2017). Alterations in the medial PFC may therefore, also influence how rats behave on this task.

The significance of these three behaviour stategies is that they represent behavioural hallmarks of DLS-mediated control versus VS control and mPFC influence. In addition, chronic stress could induce neuroplastic modifications in these striatal and frontal regions and therefore, influence choice strategies.

2.6 Stress and decision-making

Chronic stress is thought to alter decision-making through the structural reorganization of neural circuits leading to different decision-making strategies (figure 1) (Dias-Ferreira et al., 2009; Morgado, Sousa, & Cerqueira, 2015). This occurs through atrophy in prelimbic regions of the PFC and dorsal medial striatum (DMS) shifting behaviour towards a DLS response (Dias-Ferreira et al., 2009).

Chronic stress therefore, could cause hypertrophy within the DLS and increase habitual responding or stimulus-response choices (Dias-Ferreira et al., 2009; Skelin et al., 2014). Since the medial PFC might be effected, deficits in behavioral flexibility, response inhibition, and impairments in working memory could also be seen (Cerqueira et al., 2007; Daw et al., 2005). Atrophy within the medial PFC could also decrease novelty seeking or exploration which is associated with EFS (Gruber et al., 2017). The OFC which, may display hypertrophy after chronic stress, is also critical in predicting rewards based on stimulus-response associations and reversal learning (Dias-Ferreira et al., 2009; Kepecs, Uchida, Zariwala, & Mainen, 2008; Lapiz-Bluhm, Soto-Piña, Hensler, & Morilak, 2009). This however, is not definitive since hypertrophy or atrophy in the OFC appear mixed between stress studies (Morgado et al., 2015). In addition, glucocorticoids enhance dopamine release within the VS which could account for how acute stress may increase

drug craving (Sinha, 2008). The VS provides a limbic-motor interface for the stress response (Sinha, 2008). Repeated activation could therefore, explain why amphetamine and cocaine administration show similar patterns of habit formation and increased DLS tone as with chronic stress (Dias-Ferreira et al., 2009; Ostroumov et al., 2016; Robinson & Kolb, 1997; Sinha, 2008; Wong et al., 2017).

From this information, it can be inferred that chronic stress will change many behaviours. These include behaviours in the competitive choice task such as, win-stay, lose-shift and EFS behaviors; along with behavioural flexibility and reversal learning in modified competitive choice task paradigms. In addition, it can be inferred that chronic stress may influence anxiety and despair like behaviors in stress related tests. In the following section, I will state my hypothesis on how chronic stress might alter frontal and striatal brain regions and their corresponding behavior.

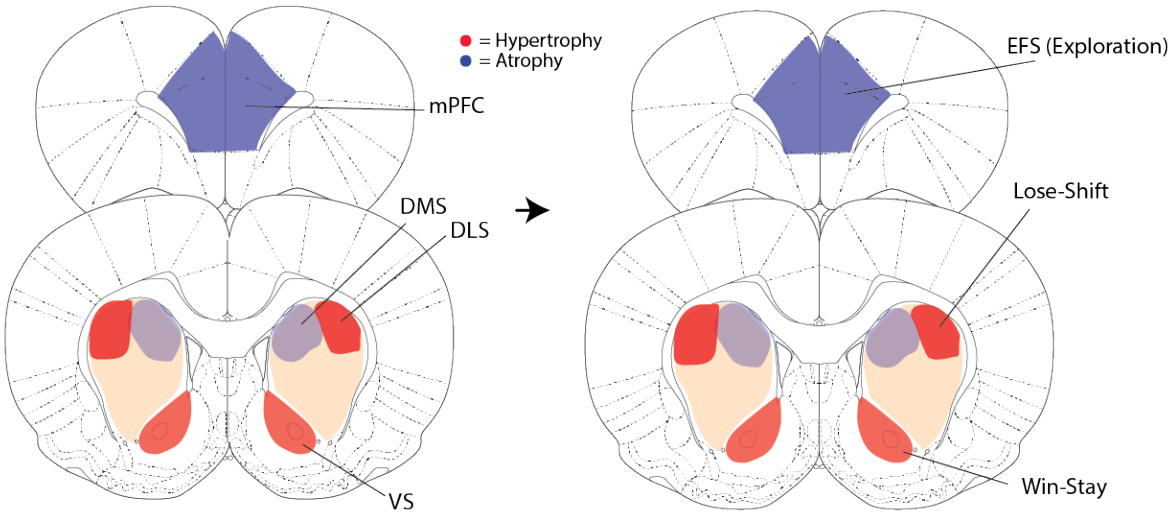

Figure 1. Frontal and striatal brain regions impacted from chronic stress exposure. Chronic stress causes atrophy within the medial prefrontal cortex (medial PFC) and dorsal medial striatum (DMS)

while causing hypertrophy within the ventral striatum (VS) and dorsal lateral striatum (DLS). For my thesis, I used a competitive choice task to measure changes in decision-making strategies which, correspond to changes in these brain regions. In this competitive choice task (CCT), rats are trained to compete against a computer algorithm to maximize reward (sucrose water) delivered in two adjacent wells after initiating a nose-poke at a central odor port (figure 3). The decision-making strategies in this task are win-stay, lose-shift and extraneous feeder sampling (EFS). Win-stay is the response when the rat returns to the same reward well after the previous trial. This is thought to be controlled by the ventral striatum. Lose-shift responding occurs after a rat does not receive a reward at the reward well and will choose the opposite well during the following trial. This represents habitual or stimulus-response based decisions, representing activity within the DLS. Extraneous feeder sampling (EFS) is the phenomenon where after either receiving or not receiving a reward, instead of initiating another trial, they will inspect the other reward well. This is theorized to be associated with novelty or exploration and might involve the medial PFC.

2.7 Theory

Below, I have summarized how I believe chronic stress will alter rodent behavior on the forced swim test, open field test, and the competitive choice task.

Theory: The brain theory states that all behavior is a product of brain function.

It follows that both normal behaviour and abnormal behaviour are products of brain activity. Therefore, chronic stress will produce its effects on behavior by altering neural structure. Independent evidence suggests that these alterations may take place in cortical and striatal brain regions. You could say in short if one considers the brain as a target of stress then per Selye's general adaption syndrome, the brain should show an alarm to stress, then resistance, then finally exhaustion to stress (Selye, 2013). These changes in principal, can be detected by formal behavioural assays. This is one goal of the present thesis.

Hypothesis 1: Chronic stress will sensitize the ventral striatum through repeated activation and cause long-lasting hypertrophy. These neural modifications may increase win-stay behaviour in the competitive choice task.

Hypothesis 2: Chronic stress will cause atrophy within the dorsal medial striatum and hypertrophy within the dorsal lateral striatum and therefore, shift behavioral strategies from action-outcome responding towards stimulus-response choices. This will be reflected in the competitve choice task as increased lose-shift behaviour.

Hypothesis 3: Chronic stress will cause neural modifications within pleasure and fear related regions in the brain including but not exclusive to: the amygdala, thalamus and frontal cortex. This may be shown as increased anxiety and despair in the forced swim test and the open field test. Also, rats might display anhedondia from reduced sucrose preference in the competitve choice task.

Hypothesis 4: Chronic stress will cause modifications within orbital and prefrontal regions of the cortex including atrophy within the medial PFC. These modifications may reflect deficits in reversal learning, novelty seeking (EFS) and behavioral flexibility in both the CCT and modified CCT protocols.

3. Methods and materials

3.1 Definitions

Definition 1. Extraneous feeder sampling (EFS) is the phenomenon where after going to a feeder well and being rewarded or not, the rat will inspect the other well instead of initiating a new trial. This is theorized to be associated with novelty and exploration, and thought to be influenced by the medial PFC (Gruber & Thapa, 2016).

Definition 2. Win-stay responding is the behaviour displayed by the rat when it returns to the previous reward site. This is thought to be controlled by the VS (Gruber & Thapa, 2016).

Definition 3. Lose-shift responding occurs when the rat chooses the opposite well after not receiving a reward during the previous trial. This represents habitual or stimulus-response based decisions, representing activity within the DLS (Gruber & Thapa, 2016; Wong et al., 2017).

Definition 4. Inter trial interval (ITI) is the time it takes for the rat to initiate another trial after selecting a reward well (figure 3).

Definition 5. Response time is the time it takes for the rat to select a reward well following inicitaion of the trial (figure 3).

3.2 Ethics statement

All experimental procedures regarding animal use were approved on behalf of the Canadian Council on Animal Care (CCAC) and the University of Lethbridge Animal Welfare Committee.

3.3 Animals

Thirty-six long-evans adult male rats (control = 18, treatment = 18) weighing 300-450g were subjected to chronic stress at 12 weeks of age or were left undisturbed. Rats were born and raised within the vivarium at the Canadian Center for Behavioural Neuroscience. Parents consisted of colony bred and imported rodents from Charles River Inc., Sherbrook Quebec. After weaning, rats were placed in a standard shoe-box cage environment with another member of their litter. Before the stress procedure, all rats had *ad libitum* access to food and water. Control rats were guaranteed *ad libitum* access until behavioral training and testing.

Due to housing constraints, stressed and control animals were housed within the same vivarium, however, to minimize bystander stress, control animals were relocated to opposite ends of the room.

3.5 Stress procedure

The chronic model of stress that we used is an unpredictable chronic stress paradigm. Instead of using home cage modifications or alterations in light-dark cycles, we used a combination of restraint, elevated platform stress (1m x 21 x 21 cm), food restriction, water restriction, and isolation at scheduled but seemingly random times during the week

for 5 weeks (figure 1). The times and duration of each stressor were repeated every week, however, scheduled to appear unpredictable and reduce habituation. For food restriction, water restriction and isolation, rats were separated and either placed in a separate cage or remained in their home cage. Elevated platform and restraint stress took place in a separate testing rooms. Control rats were left undisturbed during this procedure with exemption to regular handling.

Time	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
7:30am		Restraint (1h)		Elevated Platform (1h)			
10:00am	Elevated Platform (30min)		Collect feces for Corticosterone ELISA				
12:00pm					Restraint (1h)		
2:00pm		Elevated platform (30min)	Restraint (1h)		Elevated Platform (1h)		Elevated platform (30 min)
4:00pm	Elevated Platform (30min)			Restraint (30 minutes)			Restraint (1h)
6:00pm			Restraint (20 min)	Food Restriction (18h)	Isolation (24 h)	Water restrict (20h)	
7:30pm	Overnight Isolation		Overnight Isolation				Water restrict overnight

Figure 2. Weekly schedule of repeated stress treatments. A combination of mild to moderate stressors including: elevated platform, restraint, isolation, food and water restriction were randomized and repeated every week. This was done to simulate an unpredictable mild model of chronic stress. The schedule is shown above with a maximum of three stressors occurring within 24 hours. Feces were collected every Wednesday morning between 10-12am.

3.5 Open field and forced swim test

To quantify levels of distress and anxiety, rats were tested on the open field test (OFT) and the forced swim test (FST). For both procedures, rats were first put in the testing room for at least 1 hour to habituate. The OFT (120 x 105 cm) was executed by placing the rat in the middle of the field for 10 minutes and was recorded using a portable webcam (QuickCam Utravison SE, Logitech, Lausanne Switzerland). This experiment was also repeated with the addition of fox odor (2,5-dihydro-2,4,5-trimethylthiazoline, Sigma-Aldrich, St. Louis, US). Rearing, distance travelled and time spent in the center, middle and edges were examined using specialized software (Ethovison XT, Noldus, Wageningen, The Netherlands) and manual scoring. The center (30 x 20 cm) was marked 45 cm from the length, and 42.5 cm from the width of the field. The middle (75 x 61.3 cm) was similarly marked 22.5 cm and 21.25 from the centers length and width. The forced swim test was executed in a plexiglass cylinder (50 x 10 cm) for 7 minutes with only the last 5 minutes examined. Immobility was manually scored by a fellow researcher blind to treatment conditions.

3.6 Competitive choice task

Behavioral testing was performed in aluminum operant chambers (26 × 26 cm) containing two cue lights and a central port flanked by two sucrose delivery feeders (Skelin et al., 2014). The central port and sucrose feeders contained infrared sensors to detect entry and exit and are sensitive enough to detect the amount of licking (figure 3). For behavioral testing, animals were placed in the operant chamber for 45-minute sessions. Control of the task was automated by an arduino mega microcontroller (Digi-key Electronics, Thief River Falls, Minnesota, USA) receiving commands via custom software on a computer.

Illumination of the cue lights indicated the beginning of a new trial, signaling the animal to nose-poke in the central port. A tone (6 kHz; 150 ms duration) then prompted the animal to select one of the two sucrose delivery feeders. If the correct feeder was chosen, a reward (60 μL of 10% sucrose solution) was delivered after a 0.5 second delay. If the incorrect feeder was chosen, no sucrose was delivered.

Once a feeder was chosen, or if no feeder was chosen in the 15 seconds following a nose-poke, the trial ended, and the animal had to return to the central port to initiate a new trial. In the first session of behavior shaping, animals were rewarded upon every feeder entry following a nose-poke in the central port. This was to train them to perform the nose-poke and feeder entry sequence. In the second session, the probability of reward was 50% for each feeder entry following a nose-poke, to train them to learn that not all responses lead to reward. In all subsequent sessions, reinforcement was controlled by an algorithm that attempted to minimize the number of rewards given to the animal by predicting which feeder it would select. This was done by examining the choices and reinforcements from the previous four trials. If either feeder were selected at a higher than chance rate, it would be unrewarded for the upcoming trial.

The task thus implements a two-player competitive choice task and selects for rats to choose as randomly as possible. Over consecutive days of training, two small (4.0 cm), medium (8.5 cm), or long (13.5 cm) parallel barriers were added to the operant chamber to separate the central nose-poke port and the feeders. This reduced feeder bias from body orientation, by promoting posture that was orthogonal to the wall in which, the port and feeders were mounted. Rats were trained until they completed two consecutive sessions of at least 150 trials with the long barriers. This criterion was met by training session 13 for

all rats in the study. All subsequent training and testing sessions were run with the long barriers. Sessions that failed to reach 150 trials were omitted from analysis. Similarly, to minimize confounding variables, trials were omitted where rats would display EFS after the execution of a trial for the analysis of win-stay and lose-shift. Other than win-stay, lose-shift and EFS, response time, ITI, error rate and licking frequency before reward were also analyzed.

To quantify behavioral flexibility and reversal learning, a newer protocol called reverse probability blocks was utilized. Using the same CCT apparatus, sessions were broken up into blocks containing 40 trials and reward probabilities 80% and 20% were assigned to each reward feeder for that block. After each block, probabilities were reversed such that the rat must associate the higher reward probability with the opposite feeder. Error values were then further compared to assess behavioral flexibility along with, EFS, ITI, response time and licking. Both CCT and the reverse probability blocks data were analyzed using Matlab version 2016a (Mathworks, Natick, Massachusetts, USA).

3.7 Statistics

Statistical tests were executed using Prism version 6 (GraphPad La Jolla, California, USA). Before all ANOVA and unpaired t-tests, D'Agostino-Pearson omnibus normality test was performed to confirm data was normally distributed. An unpaired t-test was used to determine group differences between the FST, rearing in the OFT and corticosterone assays. For the OFT, a two-way ANOVA was used to determine group differences between center, middle and edge regions. For the CCT, a two-way repeated measures ANOVA was used to test differences between treatment groups accounting for differences and time for

averaged testing sessions. Also, win-stay and lose-shift values were plotted against ITI for each subject, and group values were averaged for each time bin. Linear and non-fit second polynomial regressions were used to compare differences between curves as stated in (Gruber & Thapa, 2016).

A Two-way repeated measures ANOVA confirmed regression results. A Two-way ANOVA was used for the reversal probability blocks task in the comparison between the high and low probability blocks. This was also executed for the OFT between distance, and duration spent between the edges, middle, and center regions. A unpaired t-test was performed on the FST, rearing, and animal weight. Alpha level of 0.05 was used for all for all statistical tests.

3.8 Feces extraction

Feces were collected (2 pellets) each week during the stress procedure and stored at -80ºC. Fecal matter was dried in an oven overnight at 40ºC to remove water and then ground with a mortar and pestle. After sifting through a stainless-steel mesh, fecal matter was weighed, and 200mg was used for analysis. Ethanol (1ml) was added to the fecal sample and thoroughly mixed for 30 minutes. Samples were then centrifuged (Thermofisher Scientific Inc, Heraeus Multifuge X3, Waltham Massachusetts, USA) at 5000 rpm for 15 minutes. The supernatant (1.5ml) was extracted, and ethanol was then dried off using a centrifugal evaporator (Thermofisher Scientific Inc, Savant SpeedVac, Waltham Massachusetts, USA) and stored at -20. Detailed procedures can be found on the website: https://www.arborassays.com/assets/Steroid-Solid-Extraction-170921.pdf.

3.9 Corticosterone assay

All materials were placed at room temperature for at least 2 hours to ensure optimum enzyme activity. Ethanol (100 µL) was added to dried fecal extract followed by a 1:10 dilution with buffer. Corticosterone concentrations were detected using enzyme-linked immunosorbent assay (Arbor Assays, LLC Ann Arbor, Michigan, USA). Wells were washed (BioTek EL50 microplate washer, BioTek Inc., Vermont, US) and results were obtained using a microplate reader (BioTek Synergy HT, BioTek Inc., Vermont, US). Intra and inter-assay precision were calculated to be 6.62% and 7.68%.

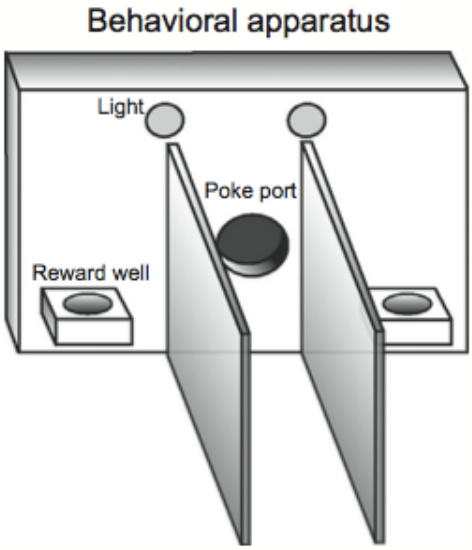

Figure 3. Behavioral apparatus for the competitive choice task. Rats are trained to do a central nose-poke and choose between either left or right wells. Probability of reward is based on a computer algorithm that accounts for the rat's previous choices hence, to maximize reward the rat needs to choose as randomly as possible. Within this task, it is thought that behaviors are represented by specific brain regions. These include win-stay (VS), lose-shift (DLS) and EFS (medial PFC). Using a different protocol called the reverse probability blocks task, where, high or low probability are assigned to each well and then reversed, reversal learning and behavioral flexibility can be quantified.

4. Results

4.1 Stress tests

After the chronic stress administration, animals were tested on the FST and OFT and retested on the OFT with the presence of fox odor. Stress caused no effect on immobility in the FST ($t(34) = 0.1937, p = 0.8475$; figure 4). Similarly, no trend was seen for the OFT with distance travelled in each zone ($F(1,102) = 0.0203$, $p = 0.8869$; figure 5A), time spent in each region ($F(1,102) = 0.6057$, $p = 0.4382$; figure 5B) and rearing ($t(34) = 0.6556$, $p = 0.5165$; figure 5C). When retested on the OFT with the presence of fox odor, there was a very significant effect on distance ($F(1, 102) = 20.87, p < 0.0001$; figure 6A) where stressed rats moved more along the edges than controls ($t(102) = 6.982$, $p < 0.0001$; figure 6A) as well as reared more ($t(33) = 4.369, p = 0.0001$; figure 6C) . No difference however, was seen between the time spent in zones ($F(1,102) = 0.0288$, $p = 0.8656$; figure 6B).

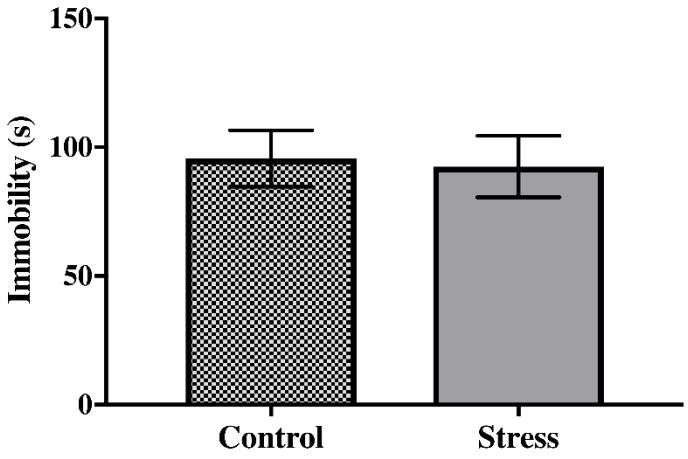

Figure 4. Results of the forced swim test. The graph shows the total time (seconds) rats spent floating (+/-SEM) between cohorts. Stressed rats showed no difference in immobility compared to controls $t(34) = 0.1937$, $p = 0.8475$.

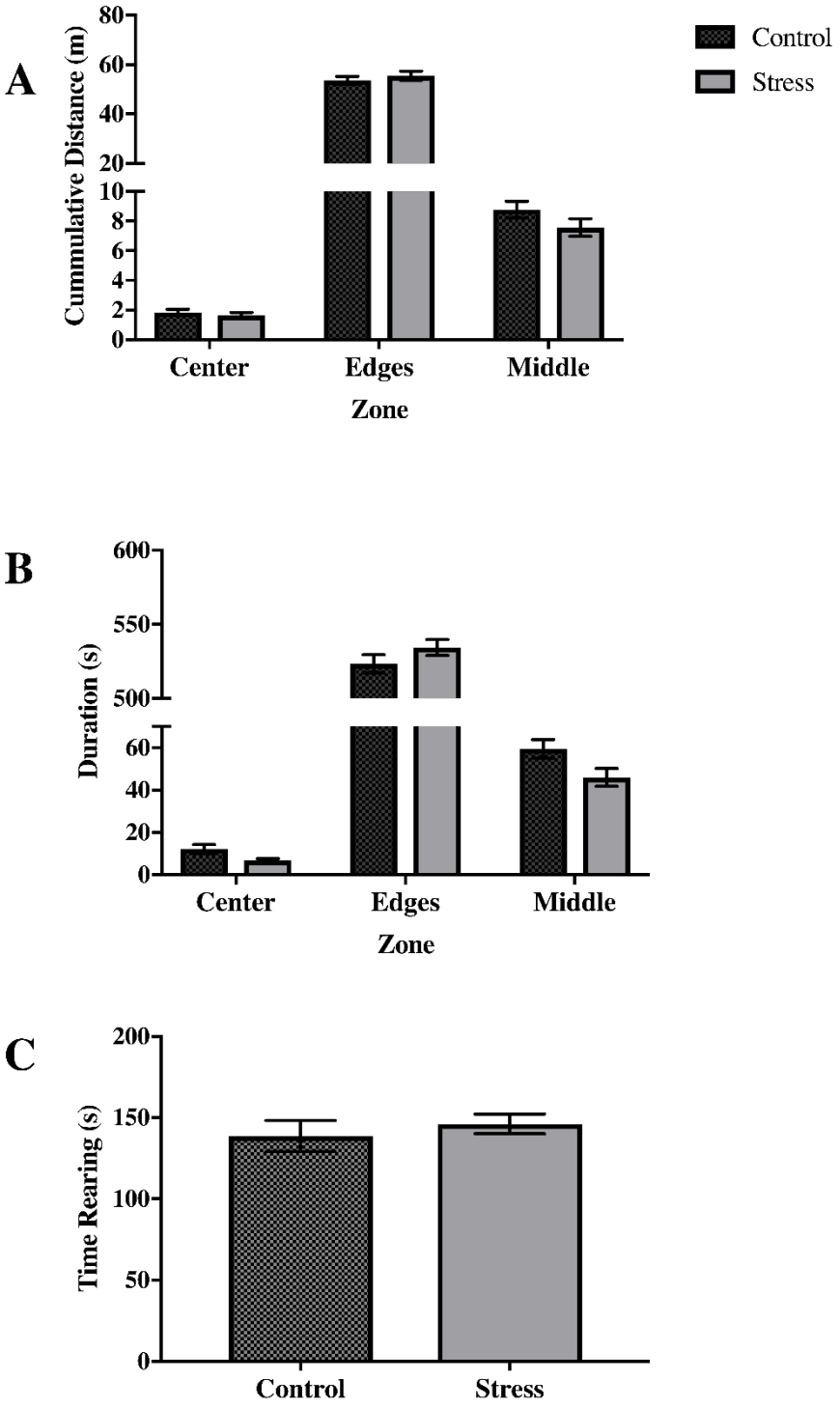

Figure 5. Results of the open field test. Cumulative distance (meters) and duration(seconds) (+/- SEM) is plotted between edges, center and middle regions. Total time spent rearing (seconds) is also displayed. A) No significance was seen between treatment groups ($F(1,102) = 0.0203$, $p = 0.8869$) in either edges, center and middle regions for the distance animals travelled. B) Stressed and controls also spent identical times in edges, middle and central regions ($F(1,102) = 0.6057$, $p = 0.4382$). C) Stressed rats displayed identical rearing than controls ($t(34) = 0.6556$, $p = 0.5165$).

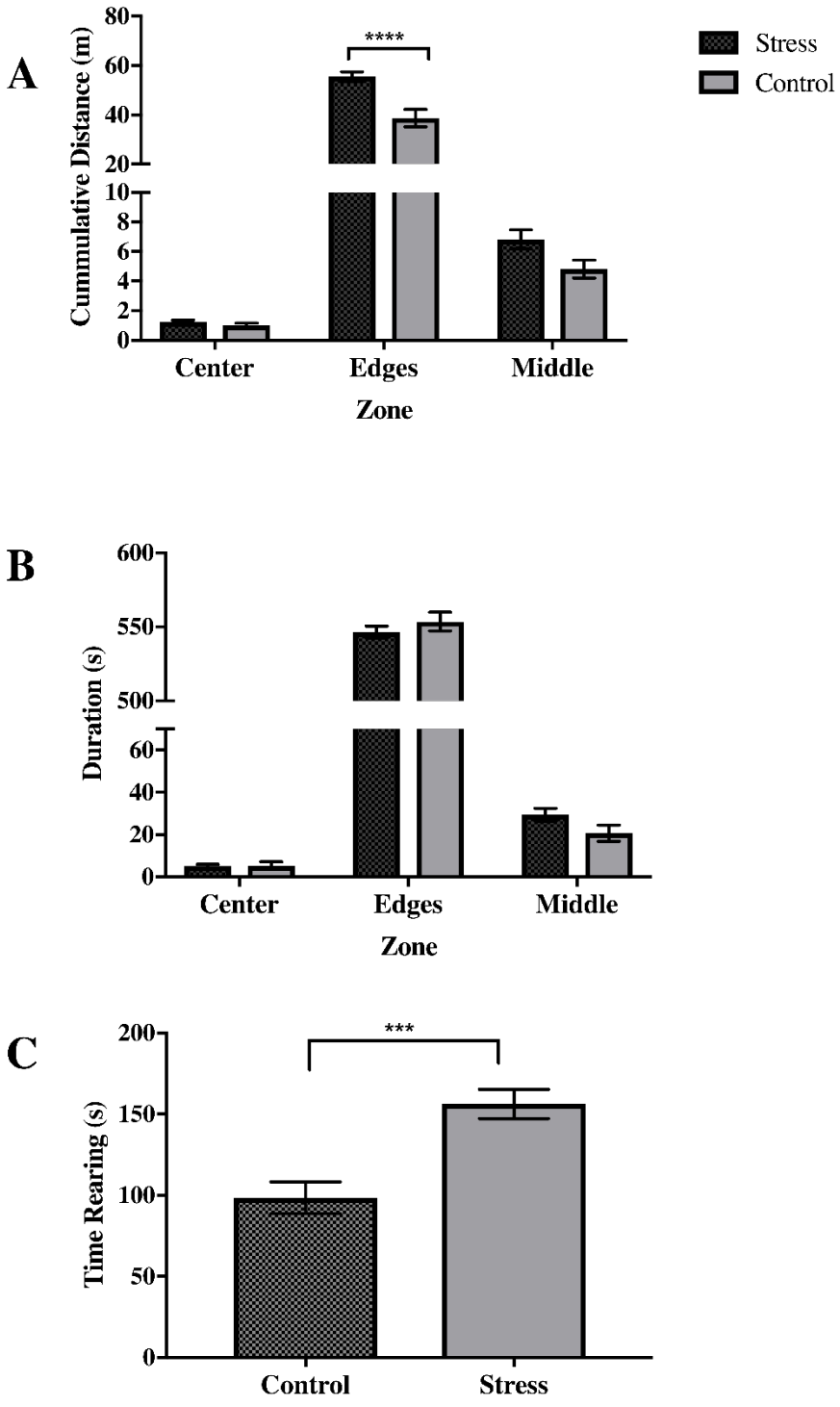

Figure 6. Results of the open field test paired with fox odor. Cumulative distance (meters) and duration (seconds) (+/-SEM) is plotted between edges, center and middle regions. Total time spent

rearing(seconds) is also displayed. A) With the addition of fox odor, there was an highly significant effect on movement ($F(1, 102) = 20.87, p < 0.0001$), whereby, stressed animals traveled a longer distance ($t(102) = 6.982$, $p < 0.0001$). B) No difference was seen between times spent in edges, middle and center zones ($F(1, 102) = 0.0288$, $p = 0.8656$). C) Stressed rats also displayed more rearing ($t(33) = 4.369, p = 0.0001$). ***p < 0.001, ****p < 0.0001

4.2 Competitive choice task

After thirteen days of continuous training on the competitive choice task, six testing days were averaged together, and the results were grouped into 3-minute time values. Differences in group mean, accounting for time, was processed through a two-way mixed-model repeated measures ANOVA (see methods). A significant difference was seen in speed between stressed and controls (figure 7). ITI and response time values were significantly lower in stressed rats than controls ($F(1,31) = 20.40$, $p < 0001$; figure 7A) ($F(1,31) = 11.31$, $p = 0.0017$; figure 7B). Counter to my hypothesis, no significance was seen between win-stay ($F(1,31) = 1.43$, $p = 0.2403$; figure 8A) and lose-shift ($F(1,31) = 0.25$, $p = 0.6173$; figure 8B).

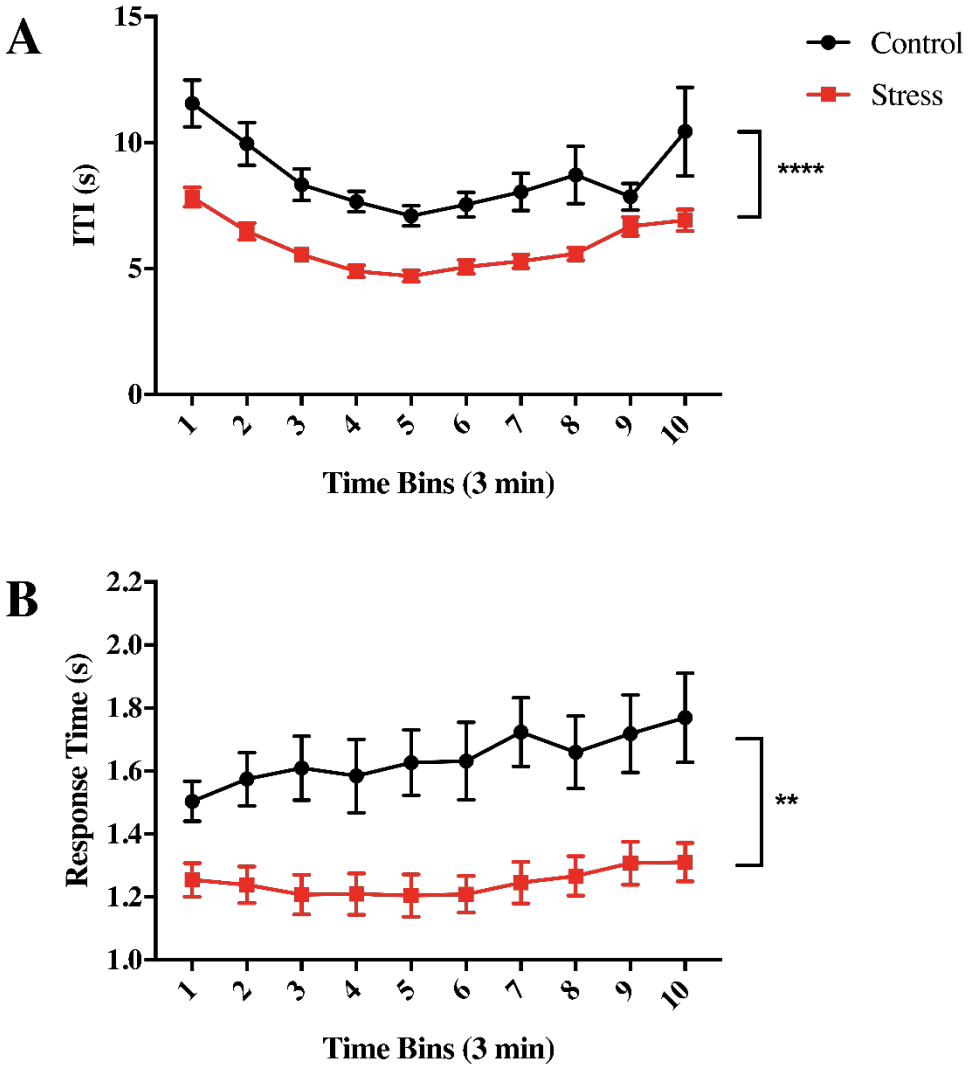

Figure 7. ITI and response time displayed from results of the CCT. Averaged times (seconds) (+/- SEM) is displayed for stressed and control rats over 3-minute time bins for all testing sessions. A) ITI values were significantly lower in stressed rats $F(1,31) = 20.40$, $p < 0.0001$. B) Response time was also significantly lower in stressed rats $F(1,31) = 11.31$, $p = 0.0017$. **p < 0.01, ****p < 0.0001

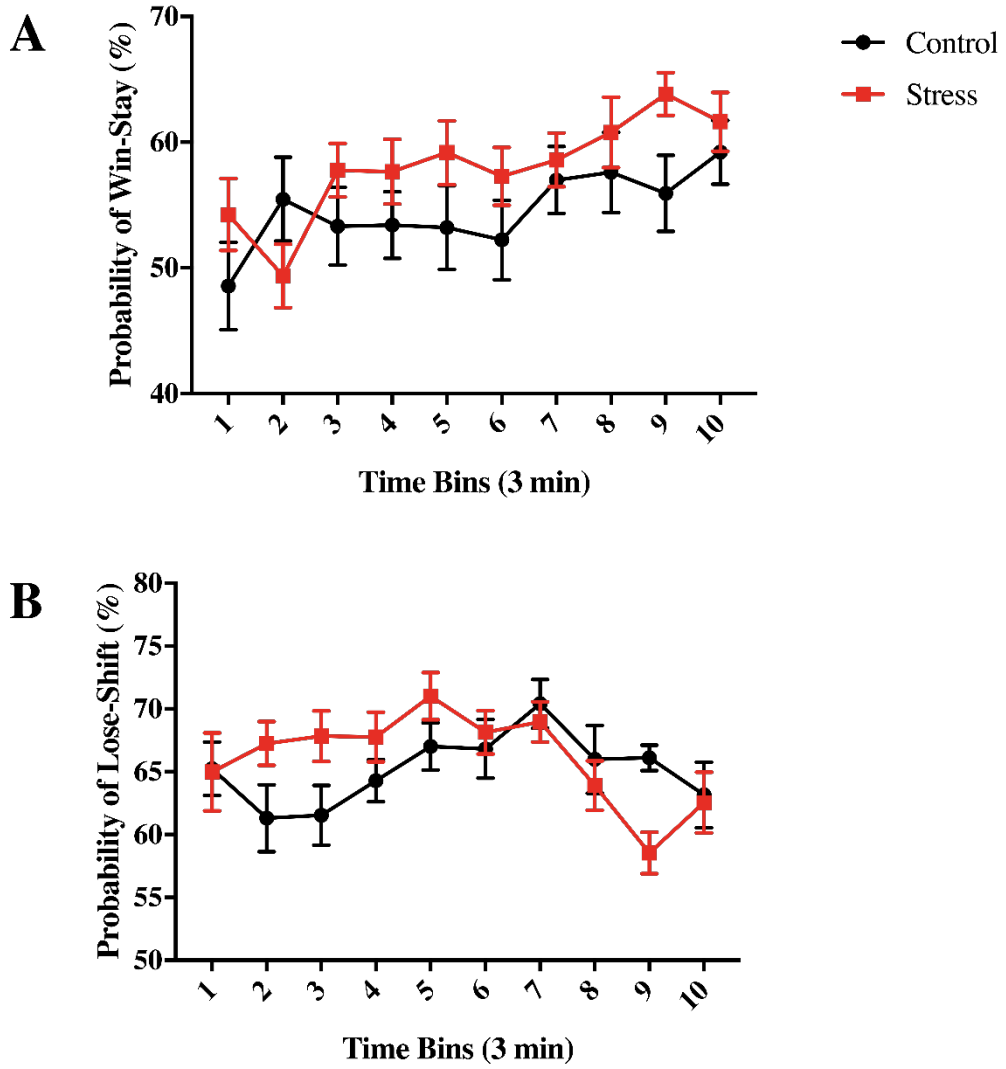

Figure 8. Probability of win-stay and lose-shift percentages from the CCT. Results are (+/-SEM) plotted against 3-minute time bins for all averaged sessions. No significance is seen in both A) win-stay ($F(1,31) = 1.43$, $p = 0.2403$) and B) lose-shift ($F(1,31) = 0.25$, $p = 0.6173$).

Considering that stressed rats were faster, and both lose-shift and win-stay show a separate and distinct time-dependent memory trace, values were examined as a function of ITI (Gruber & Thapa, 2016). Lose shift and win stay as a function of logarithmic scale ITI values were calculated for each rat and averaged across each testing session. These values were binned to specific time values to maximize samples over the linear time course explained in Gruber & Thapa, 2016. Lose-shift was analyzed through a linear regression

and win-stay through a nonlinear-second order polynomial fit (see methods). Although this type of analysis can accurately depict differences in individual curves between subjects, a drawback is that not all animals are equally represented within each ITI bin due to speed variations and lack of sample size. Therefore, only data including all subjects for lose-shift and win-stay values were extracted and analyzed through a two-way repeated measures ANOVA (figure 9B).

No significance was seen in lose-shift within the linear regression between stress rats (R^2 = 0.2834) and controls (R^2= 0.1986, $F(1,410) = 0.89$, p = 0.346; fig 9A). Also, no difference was seen between inclusively paired lose-shift values 0.39 and 0.69 shown by the dotted line in figure 9A ($F(1,31) = 2.958$,p = 0.0958; figure 9B). Win stay values for the non-fit second order polynomial regression showed a significant difference between stressed (R^2 =0.099) and control rats (R^2 =0.0436; $F(3,361) = 6.972$, p < 0.0001; figure 10A). Stressed rats displayed higher win-stay and this effect remained constant even when inclusive values between 0.75 and 0.99 were compared $F(1,31) = 4.303$, p = 0.0464; figure 10B). An opposite effect was seen in both EFS and licking. Stressed rats displayed less EFS behavior ($F(1,31) = 4.681$, p = 0.383; figure 11A) and less anticipatory licking ($F(1,31)$ = 7.146, p = 0.0119; figure 11B).

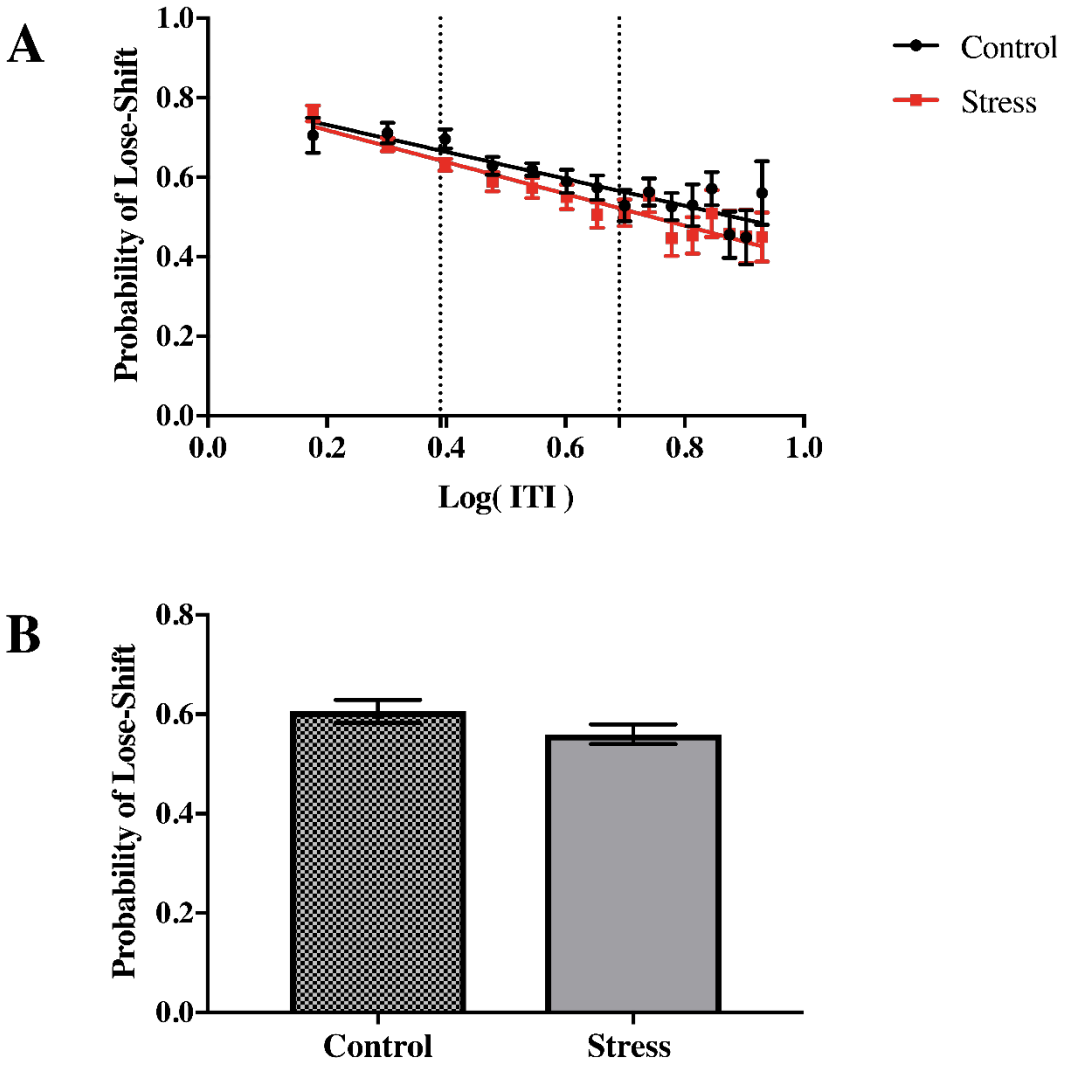

Figure 9. Lose-shift probability values as a function of ITI in the CCT. Lose-shift (+/-SEM) is plotted against log (ITI) showing a linear function. Values between 0.39 and 0.69 are averaged (+/-SEM) and plotted below. A) Linear regression was calculated for lose-shift-ITI values. No significance was seen between control (R^2 = 0.1986) and stressed rats (R^2 = 0.2834) between slopes (F(1,410) = 0.89, p = 0.3460)). B) Values between 0.39 and 0.69 were averaged and a two-way repeated measures ANOVA was calculated. Lose-shift values did not differ between groups F(1,31) = 2.9358,p = 0.0954).

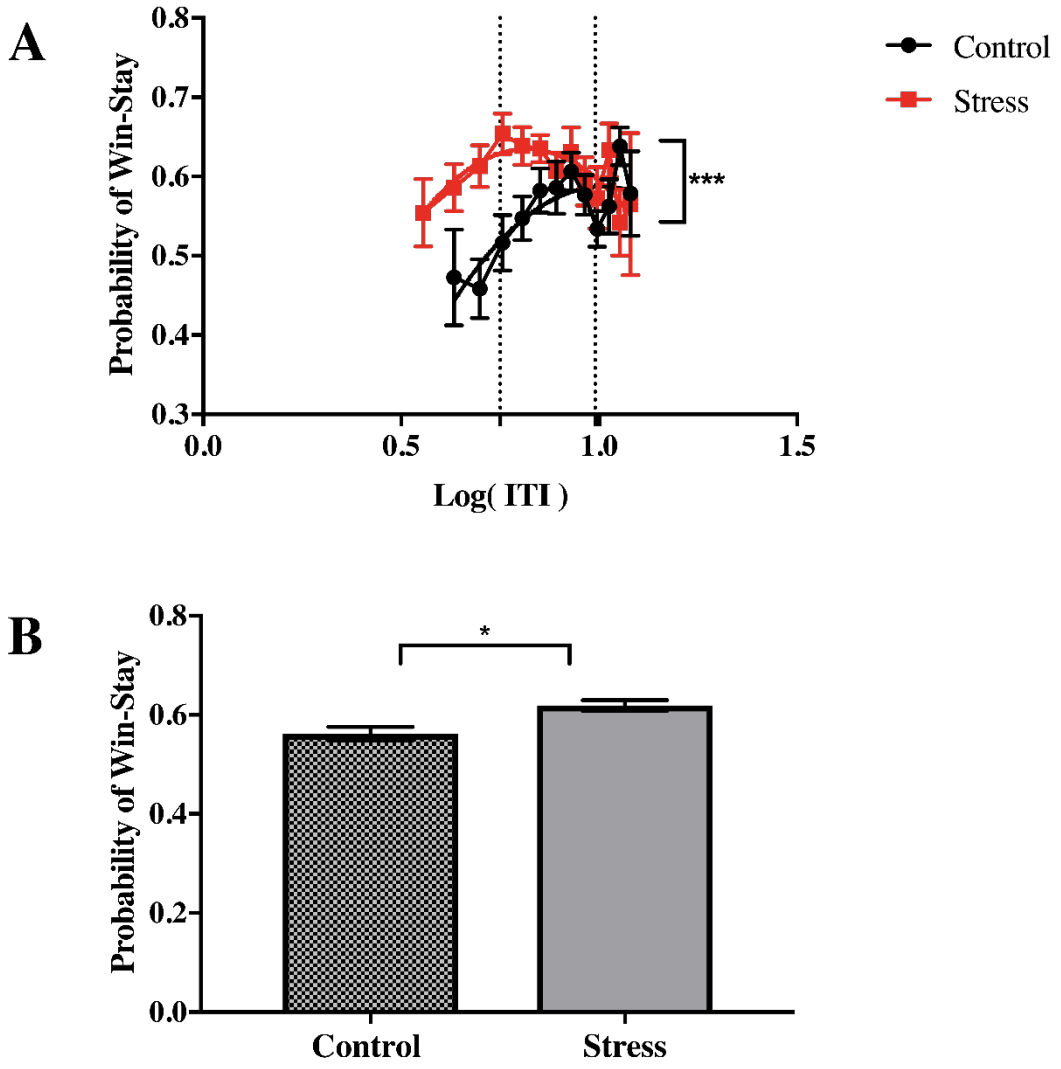

Figure 10. Win stay probability values as a function of ITI for the CCT. Win-stay(+/-SEM) is plotted against log (ITI) depicting a quadratic curve. Values between 0.75 and 0.99 were averaged(+/-SEM) and plotted below. A) A Nonlinear second order polynomial regression was calculated for win-stay-ITI values. Stressed rats (R^2 = 0.099) displayed significantly higher win-stay values compared to controls (R^2 = 0.0436; $F(3,361)$ = 6.972, p = 0.0001). B) A two-way repeated measures ANOVA was calculated for averaged values between 0.75 and 0.99. Win-stay value were significantly greater in stressed rats $F(1,31)$ = 4.303, p = 0.0464). ***p < 0.001

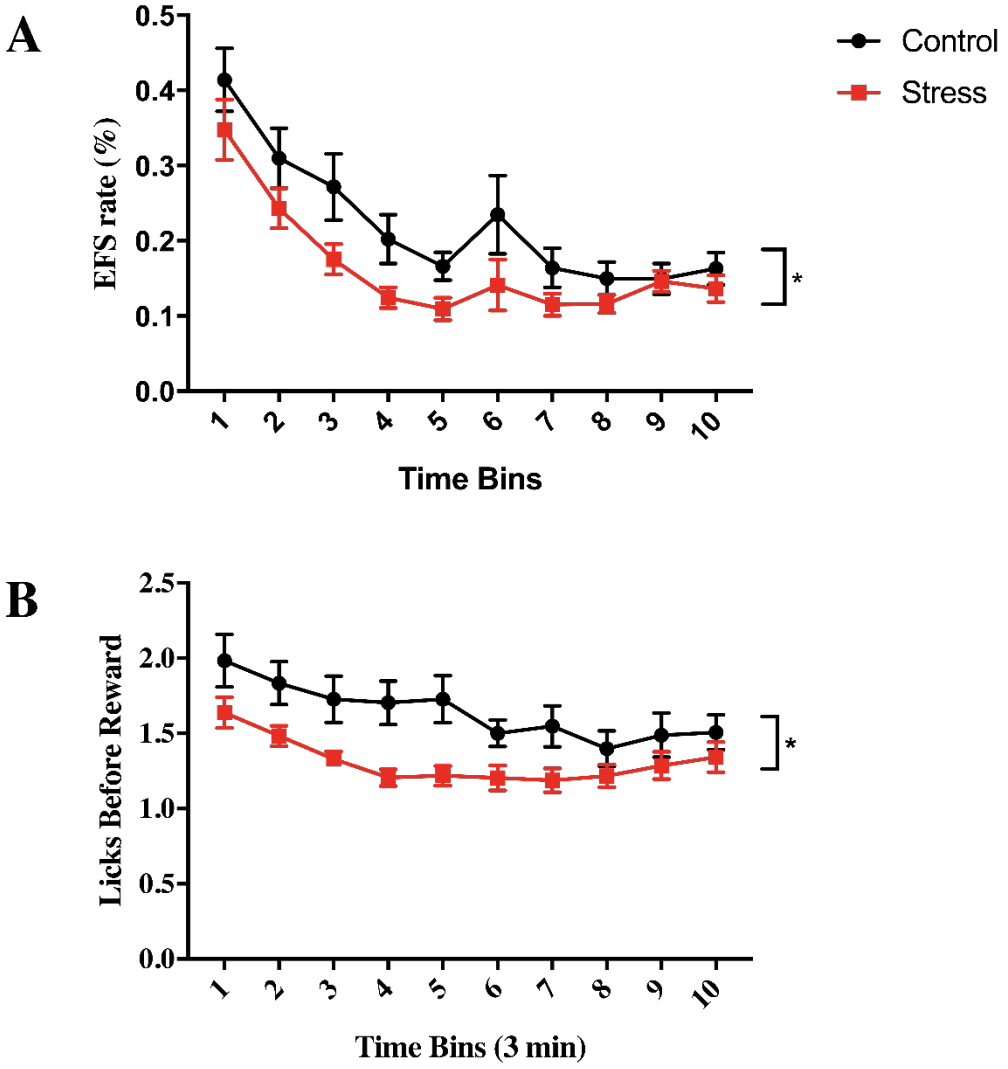

Figure 11. EFS rates and anticipatory licking before reward for the CCT. Mean EFS and licking (+/-SEM) is plotted against 3-minute time bins. A) Stressed rats displayed significantly lower EFS rates than of control animals (($F(1,31) = 4.681$, $p = 0.383$). B) Stressed rats also licked less before being rewarded ($F(1,31) = 7.146$, $p = 0.0119$). * $p < 0.05$

4.3 Reverse probability blocks task

Like the CCT, differences in ITI and response time are also seen in the reverse probability blocks task (figure 11). Stressed rats had lower ITI values ($F(1,63) = 8.853$,p =

0.004; figure 12A) at the low probability well ($t(63) = 2.831$, $p = 0.037$) but not the high ($t(63) = 1.367$, $p = 0.688$; figure 12A). Both stress and controls however, did not show significant discrepancy in ITI between high and low probability wells ($F(1,63) = 3.326$, $p = 0.073$; figure 12A). Response time was lower for stressed animals at both the high ($F(1,62) = 12.75$, $p = 0.0007$; figure 12B), ($t(62) = 2.422$, $p = 0.0007$; figure 12B) and low probability wells ($t(62) = 2.627$, $p = 0.0364$; fig 12B). Group speed differences did not differ between the high and low probability wells ($F(1,62) = 12.75$, $p = 0.2125$; figure 12B). Stress rats showed slightly but not significantly lower values in EFS behavior ($F(1,34) = 3.453$, $p = 0.0677$; figure 13A). Only control animals ($F(1,64) = 23.06$, $p < 0.0001$; fig 13A) ,($t(64) = 4.443$, $p < 0.0001$; figure 13A) showed discrimination between the high and low probability wells that was not seen in the stress cohort ($t(64) = 2.287$, $p = 0.0504$; figure 13A). Licking did not differ significantly between the stress and control groups ($F(1,62) = 2.535$, $p = 0.1164$; figure 13B). Since rats were more likely to be rewarded at the high probability well, both stress and controls licked more at the high probability well ($F(1,62) = 31.89$, $p < 0.0001$; figure 13B),($t(62) = 3.375$, $p = 0.0026$; figure 13B), ($t(62) = 4.594$, $p < 0.0001$; figure 13B).

No difference was seen in error rate between cohorts in both the reverse probability task ($F(1,64) = 2e\text{-}5$, $p = 0.9959$; figure 14A) and the CCT ($F(1,31) = 0.1911$, $p = 0.6651$; figure 14B).

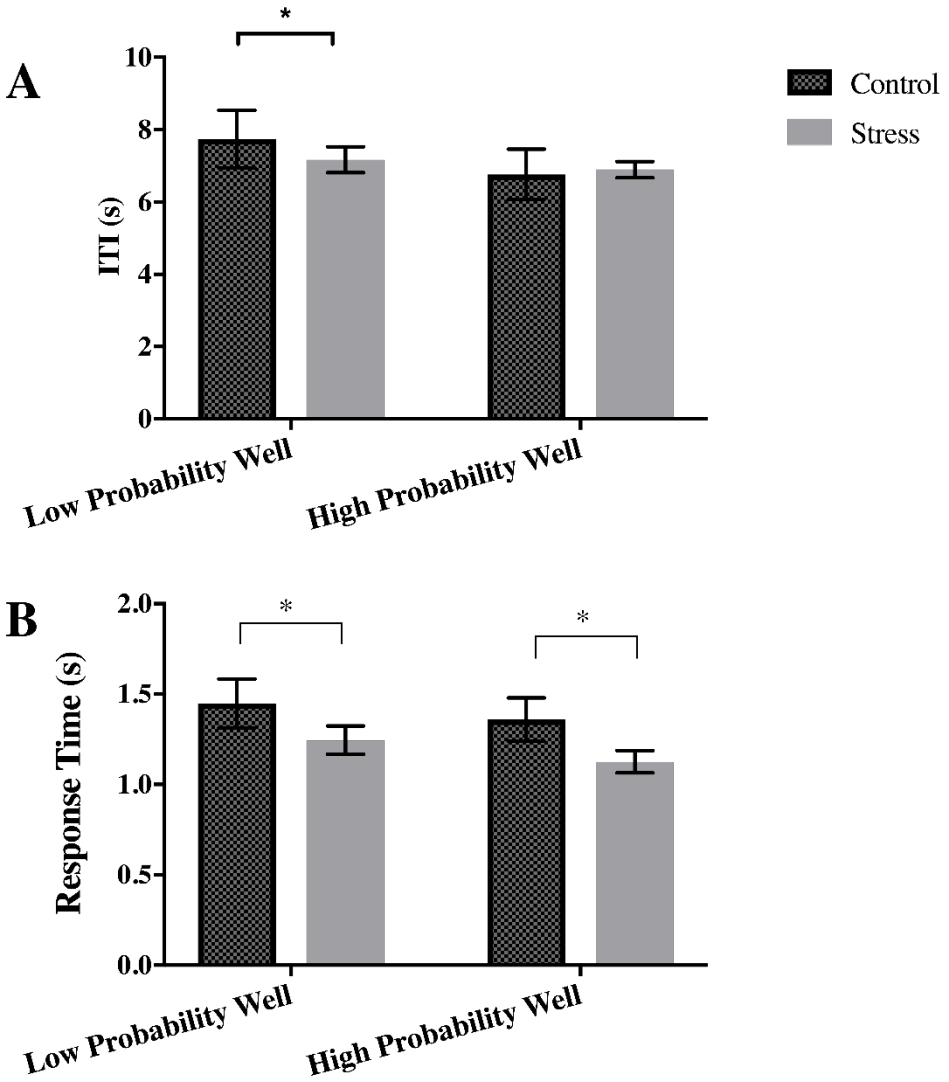

Figure 12. ITI and response time from the results of the reversal probability blocks task. Mean ITI and response time values (+/- SEM) are plotted against the high and low probability wells for both cohorts. A) Stressed rats displayed lower ITI values when choosing the low probability well compared to controls ($F(1,63) = 8.853, p = 0.004$),($t(63) = 2.831$, $p = 0.037$). Group differences were similar between the high and low probability wells ($F(1,63) = 3.326, p = 0.073$). B) Treatment rats also, had lower response times $F(1,62) = 12.75$, $p = 0.0007$) for both high ($t(62) = 2.422, p = 0.0364$) and low ($t(62) = 2.627$, $p = 0.0216$) probability reward wells. Both groups displayed similar speed for the high and low probability wells ($F(1.62) = 12.75$, $p = 0.2125$). * $p < 0.05$

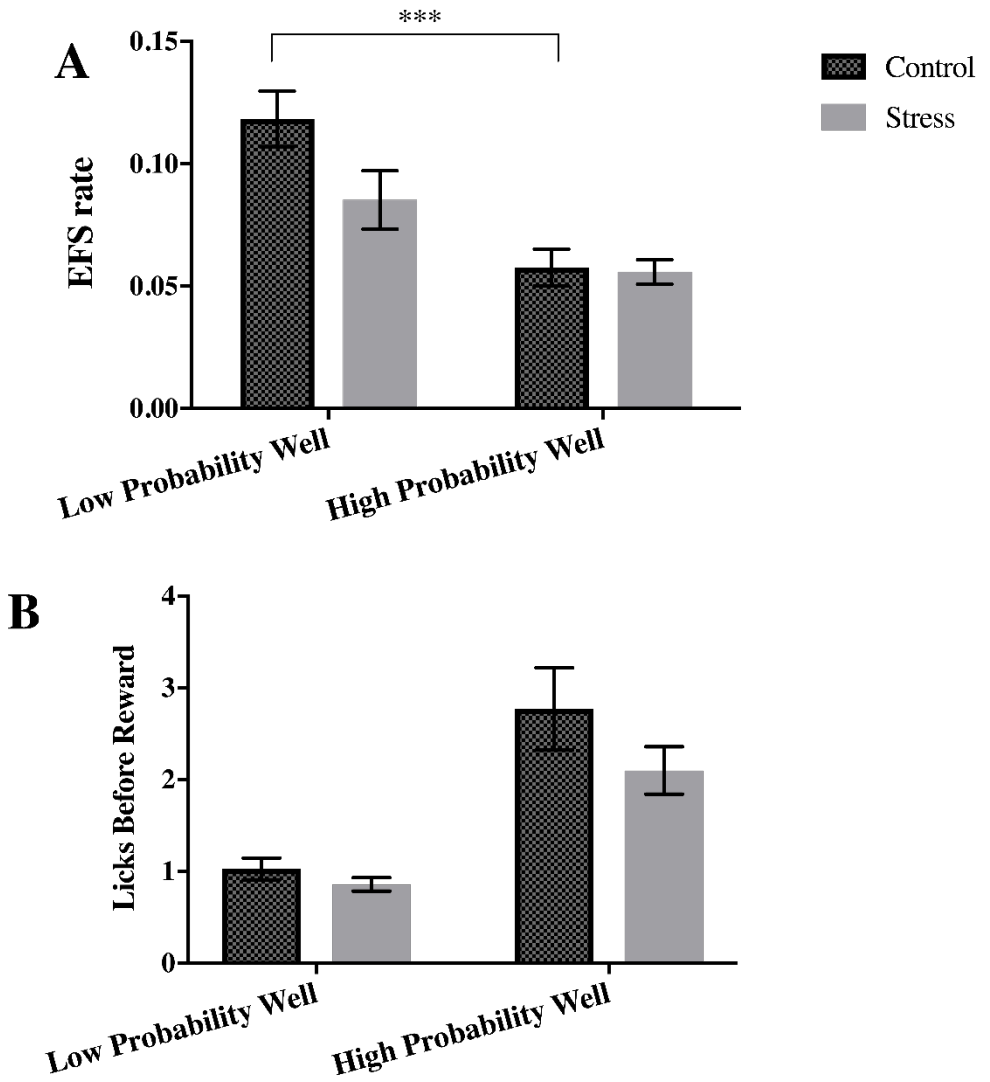

Figure 13. EFS rates and anticipatory licking from the reversal probabilities blocks task. Mean EFS and licking values (+/- SEM) for stressed and controls are displayed for the high and low probability wells. A) Stressed rats displayed slightly but not significantly lower EFS rates ($F(1,64) = 3.453$, $p = 0.0677$). Control rats showed considerable discrepancy between the high and low probability wells ($F(1,64) = 23.06$, $p = 0.0001$),($t(64) = 4.443$,$p = 0.0001$) compared to the stress cohort ($t(64) = 2.287$,$p = 0.0504$). B) No considerable difference was seen in licking between treatment groups ($F(1,62) = 2.535$, $p = 0.1164$). Both stress and controls licked more at the high probability well ($F(1,62) = 31.89$,$p < 0.0001$),($t(62) = 3.375$,$p = 0.0026$), ($t(62) = 4.594$, $p < 0.0001$). ***$p < 0.001$

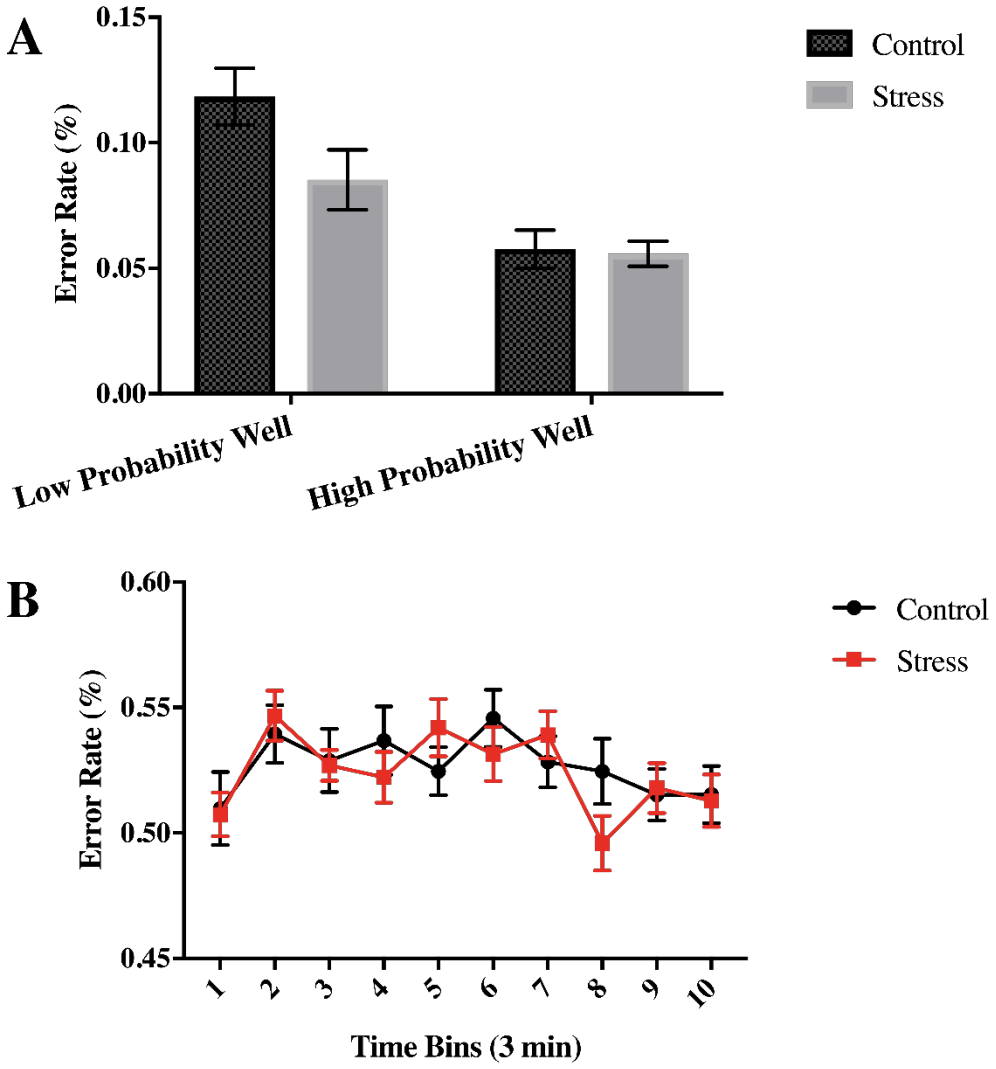

Figure 14. Error rate for the both the CCT and reverse probability blocks task. Mean error rate values (+/- SEM) is displayed for stressed and controls on both tasks. A) Stressed and control rats performed the task with equal accuracies ($F(1,64)$ = 2e-5, p = 0.9959) in the reverse probability blocks task. Both stress and control groups displayed higher error rate at the low probability well ($F(1,64)$ = 11484,p < 0.0001), (t(64) = 78.44,p < 0.0001), (t(64) = 73.33,p < 0.0001). B) Similarly, no difference in accuracy was seen in the CCT ($F(1,31)$ = 0.1911, p = 6651).

4.4 The confound of weight

In both the CCT and reverse probability blocks, stressed rats were considerably faster. After the chronic stress procedure, stressed animals were significantly smaller than

controls (t(34)=2.485, p=0.3216; figure 14) and justifiably, could move around easier within the behavioral boxes. The extent to which this was due to the stress, or from the repeated incidences of food and water restriction is unknown. To answer this question, a correlation between the last averaged six-day weight measurements and both ITI and response time values were calculated. No significant results were seen between both ITI and response time values for stress (R = -0.1783, p = 0.4936; figure 16A),(R = -0.07394, p = 0.7779; figure 16B) and control (R = 0.3389, p = 0.2166; figure 16C),(R = 0.1815, p = 0.5175; figure 16D) cohorts.

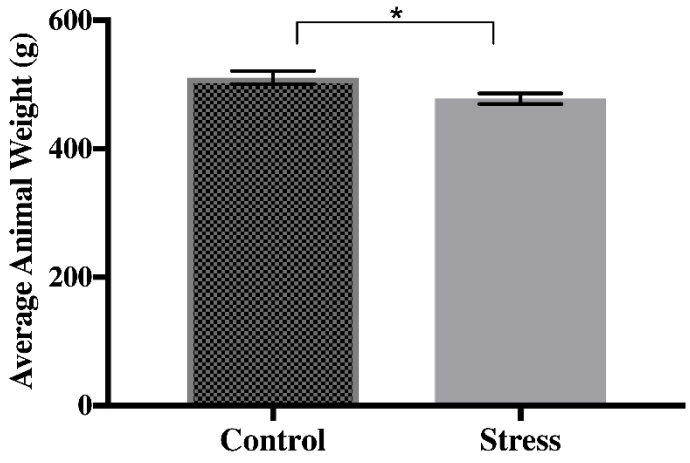

Figure 15. Averaged animal weight between stressed and controlled rats. Results are displayed (grams) (+/- SEM) above following the completion of the chronic stress treatment. Stressed rats were modestly smaller compared to controls (t(34) = 2.485, $p < 0.05$). * $p < 0.05$

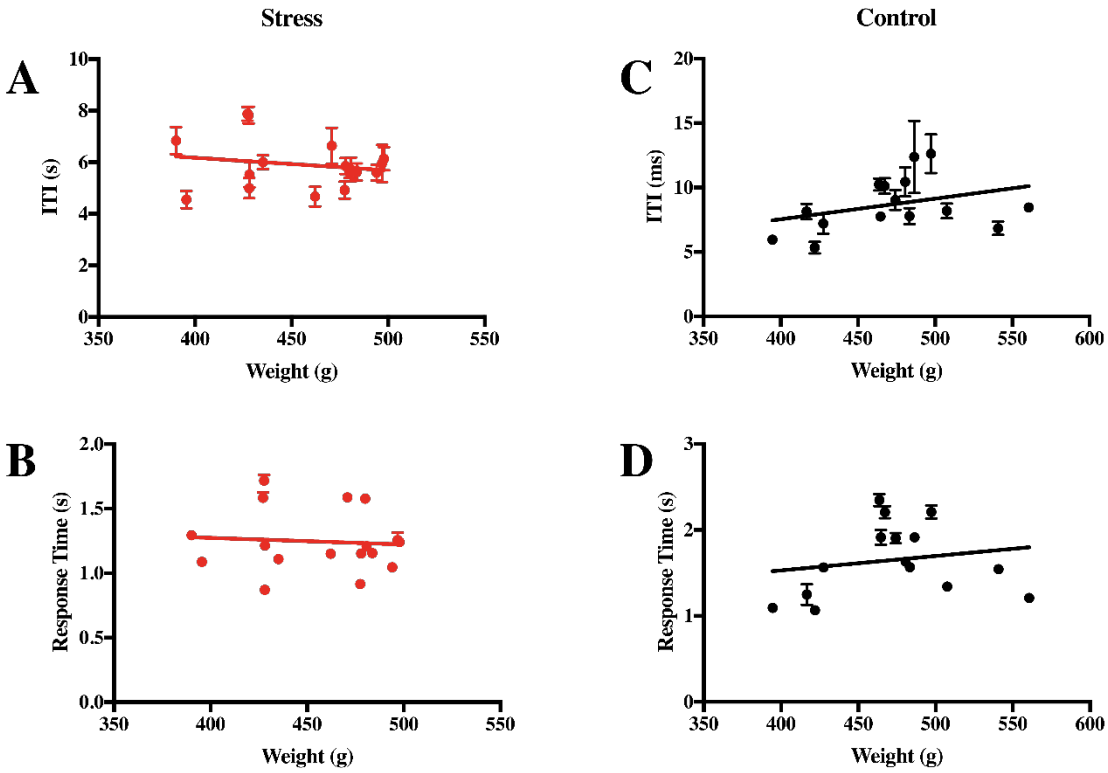

Figure 16. Correlation between performance speed and animal weight. The figure above shows the correlation of rodents averaged weight (+/- SEM) against ITI and response time values. No significant effect was seen between ITI and response times for both stress (A, B) (r= -0.1783, p=0.4936), (r= -0.07394, p=0.7779) and control (C, D) (r=0.3389, p=0.2166),(r= 0.1815, p=0.5175) cohorts.

4.5 Corticosterone

Feces were collected every week during the stress protocol for enzyme-linked immunosorbent assay (ELISA) to quantify corticosterone levels. Feces collected during the first four weeks of stress did not show significant differences ($t(30) = 0.5724$, $p = 0.6451$),($t(28) = 0.05639$, $p = 0.7842$),($t(26) = 1.108$, $p = 0.5952$), ($t(30) = 0.5724$, $p = 0.6451$) After the last week of stress, however, there was a large decrease in the stressed rat's corticosterone levels ($t(33) = 4.872$, $p<0.0001$; figure 17).

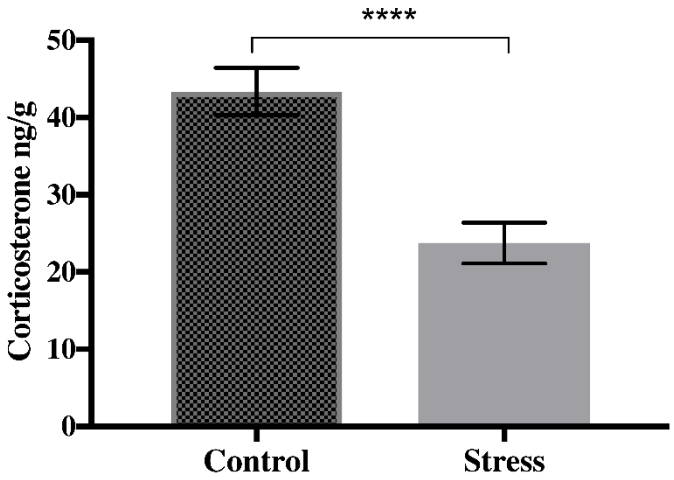

Figure 17. Fecal corticosterone after the 5th week of the chronic stress treatment. Mean corticosterone levels (+/-SEM) are shown for each cohort. After the 5th week of stress, rats had remarkably decreased levels of fecal corticosterone ($t(33) = 4.872, p < 0.0001$). ****$p < 0.0001$

5. Discussion

Stress is the response to real or perceived threats. Acute stress is beneficial for survival but chronic stress can be maladaptive. For example, chronic stress can cause brain and behavior modifications which may increase the risk for mental illness (Arnetz & Ekman, 2006). In the last 50 years, research has shown that chronic stress may lead to the development of anhedonia, despair, anxiety, and memory deficits. Only recently has it been demonstrated that chronic stress impairs decision-making and reward processing (Cerqueira et al., 2007; Dias-Ferreira et al., 2009; Koehl et al., 1999; Seckl, 2007). Based on this literature, chronic stress is believed to alter the brain in a few ways: First, chronic stress causes a shift in striatal circuit control from action-outcome strategies (DMS) to sensory-response or habitual choices (DLS) (Dias-Ferreira et al., 2009). Second, chronic stress sensitizes the VS, increasing excitation (Dias-Ferreira et al., 2009; Sinha, 2008). Third, chronic stress alters pleasure and fear related regions in the brain causing despair,

anxiety and anhedonia. Finally, chronic stress causes could cause atrophy within the medial PFC (Cerqueira et al., 2007). To measure these observations thought to be due to chronic stress, I subjected stressed rats to behavioral tests that should reflect these changes in brain structure. The results I obtained matched some of these observations but not all of them. In the following sections, I will elaborate on the results of these behavioral tests.

5.1 The forced swim test

The forced swim test is a widely-used test to measure behavioral despair. The principle behind the test is learned helplessness where the animals displays limited or no attempt to escape from stressful stimuli (Schmidt, Wang, & Meijer, 2011). Unlike most chronic stress studies, no results are seen in the FST (figure 4**)**. Studies utilizing this test, however, often pair it with antidepressants; and depending on what drug is used, can have a stimulating effect and reduce floating (Detke, Rickels, & Lucki, 1995). Room illumination is also a factor, where medium and bright lighting conditions caused stressed rats to swim more (Strekalova et al., 2005). Since room illumination during this task was not controlled, the results may not accurately reflect the stress treatment. In conclusion because no effects were seen, I will direct my attention to behavioral measures that did reflect the consequences of stress.

5.2 Open field test

Rodents have an aversion to open spaces because these pose a higher threat of predation. The OFT, therefore, is a reliable test to measure anxiety which can be measured as differences in rearing, freezing, distance traveled and differences in the time spent along

the edges vs. the center. When the animals were first tested on the OFT without fox odor, no differences were seen in any of these categories. It was not until animals were retested with the presence of fox odor that results were seen. This suggests that the previous test did not produce sufficient anxiety driven response. Stressed rats also displayed higher levels of movement, which was complemented by increased levels of rearing (figure 6). In both tests, no group differences were seen in the time spent within the center, median, and edges.

Because more anxious rats are expected to spend less time in the center compared to controls, the data would suggest no difference in anxiety between groups (Katz, Roth, & Carroll, 1981). The open field test is also often used as a test for exploration or novelty seeking (Katz, et al., 1981). The results, therefore, suggest that stressed rats were more explorative and this exploration may have been directed towards escaping from the test situation. I would argue however, that if the stressed rats were freezing less than the controls, they had more opportunity to explore. This is opposite to what was expected, because chronically stressed rats should show increased anxiety and decreased locomotion in the OFT (Katz et al., 1981; Strekalova, Spanagel, Bartsch, Henn, & Gass, 2004). Some CMS models in mice, however, had shown similar results such that there was increased locomotion however, only when the room was brightly lit (Strekalova et al., 2011; Strekalova et al., 2005). Therefore, it seems that the incidences of mixed results in locomotor activity between stress studies is likely accounted for by the wide variety of OFT tests people use (Strekalova et al., 2011). Also, because these rats were previously exposed to the OFT without fox odor, the results of the retest with fox odor may reflect differences in stress adaptation (Katz et al., 1981).

5.3 The competitive choice task

In the following sections I will describe the results obtain in the competitive choice task. These will include in order speed, win-stay, EFS and task accuracy.

5.3.1 Stressed rats were faster

Evidence from both the CCT and reverse probability blocks task showed that stressed rats were much faster in both ITI and response time. This finding can be interpreted in a few ways. First, stressed rats were faster because they spent less time licking at the reward wells (figure 11). This idea is flawed because although reduced licking could explain the lower ITI levels. It cannot account for faster response times. This can be also be seen within the reversal probability blocks task where no group differences were seen in licking, however stressed rats were still faster in both response time and ITI values.

Second, faster rats could be indicative of increased wanting or motivation for the reward. This seems contraindicative since licking itself could be an anticipatory behaviour. I argue however, that motivation for reward (faster response times) and pleasure from the reward (licking) represent two separate processes (Berridge, 1996). In addition, reduced licking in rodents is often interpreted as a symptom of anhedonia (Strekalova et al., 2004).

Wanting or motivation for a reward is sensitive to activity from midbrain dopaminergic neurons including the VS (Schultz, 1998). Chronic stress may cause sensitization within the VS, leading to increased reward seeking behavior (Sinha, 2008). Stressed rats, therefore, could be wanting the reward but not experience the same pleasure from it (Berridge, 1996). Also, pleasure in the brain is mediated through diverse brain regions thought to involve GABA and opioid signaling (Berridge, 1996). Given the

uncertainty with stress on these brain regions, more information is needed to come to this conclusion.

5.3.2. Stressed rats display more win-stay

Previous studies have shown that chronic stress could cause hypertrophy in the DLS as well as increased dopaminergic tone in the VS (Dias-Ferreira et al., 2009; Sinha, 2008). The DLS is thought to mediate lose-shift responding while, the VS mediates win-stay. It was therefore, hypothesized that chronic stress will increase both behaviors. The results obtained showed no difference in lose-shift responding and instead, higher levels of win-stay. Chronic stress may have caused neural modifications in the VS leading to increased activity and increased win-stay. The mechanism of how this occurs is believed to be mediated through glucocorticoids. Glucocorticoids upregulated by stress are thought to increase dopamine release within the mesolimbic dopamine circuit and sensitize the VS (Sinha, 2008). What is odd, however, is that when I tried to quantify corticosterone levels within stressed and control rats, the results don't reflect this conclusion. Fecal corticosterone assays did not show any differences in corticosterone levels within the first four weeks of stress. The fifth week did show results, and instead of increased corticosterone, corticosterone levels were significant lower within the stress cohort. The reason for this could be that samples were contaminated with urine. Corticosterone accumulates in feces nine hours after entering the bloodstream and for urine, five hours later (Kalliokoski, Hau, Jacobsen, Schumacher-Petersen, & Abelson, 2010). Urine also contains twice as much corticosterone than feces and therefore could have altered the results (Kalliokoski et al., 2010). In addition, the latency and amount of urination during

collection was also more substantial in the control group. Despite this variable, it could also be that rats displayed analogous symptoms to PTSD, where glucocorticoids are lower despite stress (Oquendo et al., 2003). Perhaps, a more accurate measurment of non behavioral assays of stress could have been to measure CRH along with corticosterone. This could demonstrate when and how corticosterone levels diminshed and how behavior is affected.

Although the win-stay hypothesis was proven, it was expected that increased lose-shift responding was more likely to occur due to stress-induced hypertrophy within the DLS (Dias-Ferreira et al., 2009). Previous studies have demonstrated that lesions of the DLS showed remarkable deficits in lose-shift responding (Skelin et al., 2014). This might be because these lesions also damaged the ventral lateral striatum (VLS), which may be more sensitive to lose-shift responding than the DLS (Gruber et al., 2017).

5.3.3. Stressed rats display less EFS

Complimentary to my hypothesis, EFS behavior or exploration is significantly lower in the stressed animals within the CCT. This was not seen within the reverse probabilities block task. On the contrary, stressed rats showed impaired discrimination between the high and low probability wells for EFS behaviour. Also, EFS is sensitive to reward devaluation, which is seen in the reverse probability blocks task where EFS is higher at the low probability well (Gruber et al., 2017). Given this data, I would argue, that decreased discrimination between wells in the stressed rats represents a slight degree of reward insensitivity. Other measurements within the reversal probabilities block task should reflect this.

EFS is considered an odd phenomenon because rats are never reinforced for this behaviour and yet it persists. Recent data, suggests that the medial prefrontal cortex may be involved in this behavior because this structure plays a role in exploration and novelty seeking (Gruber et al., 2017). Although some studies show opposing results, stress causes a degree of atrophy within this region and might explain deficits in EFS responding and perhaps reward insensitivity (Cerqueira et al., 2007; Daw et al., 2005). Hypertrophy in the DLS as described in other studies may also play a role (Dias-Ferreira et al., 2009). DLS lesions increased EFS responding (Gruber et al., 2017). This is theorized to be a result of competing circuits underlying behavioral control, however, more information is needed.

5.3.4. Task accuracy was unaffected

In both the CCT and reverse probability block task, no difference in error rate was seen. This was expected because even lesioned rats display similar performance (Gruber et al., 2017). Nevertheless, more pronounced results within the reverse probability blocks task were expected (Cerqueira et al., 2007). Chronic Stress causes hypertrophy within the OFC, which is an area largely implicated in reversal learning (Lapiz-Bluhm et al., 2009; McEwan et al., 2015). I therefore expected to see differences in error rate within this task, which would reflect deficits in reversal learning. There may be mild reward insensitivity because stressed rats showed decreased discrimination in EFS between the high and low probability wells. The medial PFC and DMS are regions encoding the cost-effort value of a reward (Cerqueira et al., 2007; Yin et al., 2005). Atrophy within these regions due to chronic stress, may cause behavior to become insensitive to reward outcomes regardless of training status (Cerqueira et al., 2007; Dias-Ferreira et al., 2009; Yin, Knowlton, & Balleine, 2004; Yin et

al., 2005). Although the results do not entirely reflect reversal learning and reward insensitivity, more complex reversal learning protocols might provide insightful results.

Table 1. Summary of stress treatments on brain and behavior. The table summarizes the results obtained in this study and alleged results from other studies. We found that chronic stress decreased EFS, increased win-stay and locomotion but did not affect lose-shift. This most likely correlates to hypertrophy within the ventral striatum and or dorsal lateral striatum and atrophy within the medial prefrontal cortex.

Brain Region	Theorized effects on the brain	Behavioral results from this study	Source
Medial Prefrontal Cortex	↓ spine density and dendritic branching	↓ Reward sensitivity ↓ EFS	(Cerqueira et al., 2007; Cerqueira et al., 2005; Gruber et al., 2017)
Dorsal Lateral Striatum	↑ hypertrophy	Lose-Shift unaffected	(Dias-Ferreira et al., 2009; Skelin et al., 2014)
Ventral Striatum	↑ spine density	↑ increased locomotion ↑ Win-stay	(Gruber & Thapa, 2016; T Strekalova et al., 2005)

5.4. What does it all mean?

In this thesis, I have focused my attention on the effects of chronic stress on frontal cortices and striatal regions, regions that are vital in decision-making strategies (Gruber et al., 2017). What I found was that chronic stress causes atrophy within the medial PFC while causing hypertrophy the VS (Dias-Ferreira et al., 2009; Gruber et al., 2017; Wong et al., 2017). This is based on behavioral hallmarks of the CCT, where rats displaying increased win-stay and decreased EFS. What I also found was that stressed rats were also faster and showed hints of decreased reward sensitivity. Amoung other widespread effects of stress, the dorsal lateral striatum and orbital frontal cortex might have had some involvement (Gruber et al., 2017; Lapiz-Bluhm et al., 2009). In addition, stressed rats also showed lower levels of licking, likely due to anhedonia (Deussing, 2006).

The results show that chronic stress did indeed influence decision-making. Deviations in my predictions and results may reflect the unknown extent and complexity of chronic stress on the brain (Lupine et al., 2009). This thesis demonstrates the effects of chronic stress on behaviour and decision-making in rats. The significance of this is that these results could provide insight into stress related changes in humans and particularity, stress induced mood disorders.

6. Conclusion

The theory that guided my reseach is that normal and abnormal behaviour are products of brain activity. Therefore, chronic stress will produce its effects on behavior by altering neural structure. This includes alterations in cortical and striatal brain regions that can be quantified through behavioral assays. Taken together, my results show that chronic stress did indeed alter function within striatal and frontal corticies and consequently altered decision making stradagies. My interpretation is that chronic stress caused atrophy within the medial prefrontal cortex and hypertrophy within the ventral striatum leading to reduced novelty seeking, increased reward seeking and slightly diminshed behavioural flexibilty. Stress on the brain however is highly complex and its influence isn't fully understood. In conclusion, chronic stress causes complex brain and behaviour modifications that might change decsion making strategies. This study provides a glimpse of how decision-making strategies are altered after chronic stress and how they might be altered in stress related mood disorders. Further research uncover why individuals after stressful periods, may develop poor coping skills and susceptibility towards addictions (Greenberg et al., 1999; Kessler et al., 2005).

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