Investigating the influence of Stress on Motivational Dysfunction in Male and Female Rodents

Emily Laine Errante
emilylerrante@gmail.com

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ABSTRACT

INVESTIGATING THE INFLUENCE OF STRESS ON MOTIVATIONAL DYSFUNCTION IN MALE AND FEMALE RODENTS

Emily Laine Errante, Ph.D.
Department of Psychology
Northern Illinois University, 2022
Leslie Matuszewich, Director

Motivational dysfunction is present in a variety of disorders, including major depressive disorder, schizophrenia, and Parkinsonism and can negatively impact social functioning, employment, and treatment response. Interestingly, outside stressors and stress hormones have been shown to contribute to depressive episodes in humans and depressive-like behaviors in rodents, including reducing motivated behaviors. Effort-related decision making procedures have been utilized in both humans and animals in order to examine motivational dysfunction, as it offers a subject a choice between high effort/reward and low effort/reward options, but little research has examined the effects of stress on effort-related decision making behaviors.

Moreover, almost all the research that has considered motivational dysfunction in effort-based tasks has been conducted in males, despite prior research consistently showing sex differences in the prevalence of depression and depressive-like behaviors in humans. Therefore, the current study investigated the impact of acute stressors on effort-related decision making procedures in male and female rats (Experiment 1), assessed the role of the stress peptide hormone CRF on task performance in male and female rats (Experiment 2), and determined whether acute stress influenced dopamine in the nucleus accumbens (Experiment 3). Animals were trained in an FR5 task where they could lever press for a sucrose reward or consume freely available standard lab chow. Then, animals were exposed to various acute stressors. In Experiment 1, it was found that
60 minutes of restraint stress or an injection of yohimbine reduced performance in the task, as measured by total number of lever pressing, and that the sexes significantly differed in lever pressing performance with stress. In Experiment 2, while 60 minutes of restraint stress reduced lever pressing performance similar to Experiment 1, administration of a CRF antagonist did not attenuate this response nor did exogenous CRF affect responding. Finally, in Experiment 3, it was found that there were no differences in dopamine content in the nucleus accumbens nor were there sex differences when comparing baseline to various stress conditions. Collectively, these findings contribute to our understanding of the influence of stress and sex differences on motivational dysfunction to provide better options for effort-related behaviors in populations impacted by these symptoms.
INVESTIGATING THE INFLUENCE OF STRESS ON MOTIVATIONAL DYSFUNCTION IN MALE AND FEMALE RODENTS

BY

EMILY LAINÉ ERRANTE
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A DISSERTATION SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PSYCHOLOGY

Dissertation Director:

Leslie Matuszewich
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DEDICATION

To my grandparents, Vincent and Olga Errante, for inspiring me to go into neuroscience research in the first place, and to my family for never once questioning my dreams.
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CHAPTER ONE: INTRODUCTION

Motivation is crucial to survival for humans and animals alike. Unfortunately, dysfunction of motivation is commonly associated with many disorders, such as major depressive disorder (MDD), schizophrenia, Parkinsonism (Demyttenaere et al., 2005), and drug addiction (Meyer et al., 2016). Motivational dysfunction can be incredibly debilitating because it negatively impacts social functioning, employment, and treatment response (Tylee et al., 1999). Research indicates that motivational symptoms can be especially resistant to current treatment options (Stahl, 2002; Fava et al., 2014), making this class of symptoms more challenging to treat than others. Additionally, individuals with motivational dysfunction show characteristics such as reduced engagement with rewarding stimuli and diminished activational aspects of motivation, which require effort or work to receive a reward (Salamone et al., 1994, 1997). Because the underlying mechanism of effort-related dysfunction is seen in hypomotivated behaviors, effort-related decision making tasks are particularly useful to investigate disorders that have symptoms associated with reduced motivation.

Past research has shown that rates and symptoms of depression differ between males and females. Females are at least twice as likely to suffer from depression compared to males (Altemus, 2006). Additionally, research indicates that women with depression are more likely to exhibit more severe symptoms (Marcus et al., 2008, 2005) and more likely to show subtypes of depression such as hypersomnia, weight gain, and excessive fatigue (Angst et al., 2002; Blanco et al., 2012; Marcus et al., 2005; Schuch et al., 2014). Further, sex also influences comorbidities in depression, with women having higher rates of anxiety (Schuch et al., 2014) while men have
higher rates of substance use disorder as a comorbid diagnosis (Marcus et al., 2008; Schuch et al., 2014). Animal models of depression have also demonstrated a sex difference in depressive-like behaviors. More specifically, in the forced swim test, female rats have been shown to have higher levels of immobility compared to males (Dalla et al., 2008; Drossopoulou et al., 2004), which is suggestive of depressive behavior in females. Interestingly, in a chronic mild stress paradigm where sucrose intake is the dependent measure, there is a more robust decrease in sucrose consumption in male rats compared to females (Dalla et al., 2008; Grippo et al., 2005). While we know that there are substantial sex differences present in hypomotivated behaviors, little is known about the underlying cause of these differences.

Stress has long been associated with motivational dysfunction and may provide insight into the sex differences that are seen in hypomotivated behaviors. Stress has been shown to produce depressive-like behaviors in rodents (Willner, 2005), including deficits in reward-related learning (Matthews & Robbins, 2003; Dias-Ferreira et al., 2009). Previous studies report reduced motivation in rats that have been exposed to stress (Gourley et al., 2008, 2009), indicating that stress may be a trigger for motivational dysfunction. Interestingly, the neurocircuitry underlying stress and motivation are intertwined. Mesolimbic dopamine has long been associated with motivation, as animal models have shown that the behavioral activity caused by the scheduled presentation of food pellets or other rewards is dependent upon nucleus accumbens dopamine release (McCullough & Salamone, 1992; Ahn & Phillips, 1999; Bassareo & Di Chiara, 1999). Research also has shown that there is stress-induced regulation of the mesolimbic dopamine system (King et al., 1997). Dopamine release can be enhanced or inhibited based on the intensity, duration, and avoidability of a specific stressor (Baik, 2020). Thus, motivation can be influenced by the relationship between stress and mesolimbic dopamine.
The stress peptide hormone, corticotropin-releasing factor (CRF), has been shown to influence the activity of the mesolimbic dopamine pathway and the motivation to work for food rewards (Wanat et al., 2013), suggesting a mechanism that may connect stress and motivation circuitry. CRF has been shown to decrease high effort choices for a food reward when infused into the ventral tegmental area, a structure important in the mesolimbic circuitry that contains the dopamine cell bodies (Bryce & Floresco, 2016). Studies have shown that there are sex differences in CRF receptor coupling and signaling in cortical tissue (Bangasser et al., 2010), with females showing more neuronal sensitivity to CRF compared to males. Another study found that differing levels of ovarian hormones in female rats alter the responses to CRF, which could contribute to different coping strategies to stress in males and females (Wiersielis et al., 2016). However, no study to date has tested whether or not stress and CRF regulate effort-related motivated behavior in both male and female rodents and whether this regulation contributes to the sex differences observed in motivated behaviors.

Thus, the present set of experiments sought to examine the impact of stress on male and female rodents in an effort-related decision-making task to assess motivated behaviors. Two experiments tested the overarching hypothesis that stress and stress hormones mediate sex differences observed in motivated behavior associated with effort-related choice tasks. We hypothesized that acute stress will affect female rodents to a greater extent than males because females show greater sensitivity to acute stress and the stress hormone CRF, which inhibits mesolimbic dopamine (Saal et al., 2003). We hypothesized that acute stress would reduce motivation due to its impact on the mesolimbic dopamine system (Salamone & Correa, 2012; Baik, 2020), which is the system that mediates effort-related decision making (Salamone et al., 2015). Although multiple structures are involved in the mesolimbic dopamine system, the
Motivation

General Introduction

Motivation is a critical component of all behavior in humans and other animals. Rodents enthusiastically pressing a lever in a Skinner operant box for a food reward, birds building nests and humans pursuing a mate are all examples of organisms that are said to be demonstrating motivation. Broadly, the term motivation can be used to describe what appears to be the purpose underlying a particular behavior, e.g. “hunger,” “escape” or “sex” (Kolb & Whishaw, 2001). Understanding what drives behavior is essential, as it helps to explain the ability to obtain a goal (Simpson & Balsam, 2016).

When reviewing the literature, it becomes clear that defining motivation is a difficult task. Broadly, motivation can be thought of as the processes that influence the arousal, strength, and/or direction of behavior (Arkes & Garske, 1982). From the behavioral neuroscience
viewpoint, motivation is a brain process triggered by intrinsic and/or extrinsic drivers that induce an animal or a person to move towards a goal (Volkow et al., 2017). One interesting aspect of this definition is that it allows for motivation to be a brain process and implies that it is capable of being disrupted and/or altered. This is particularly important when considering treatment of motivational impairments as it provides targets for potential treatment approaches to address the motivational dysfunction seen in various disorders.

**Classical and Operant Conditioning**

In order to test treatment approaches for motivational dysfunction, it is imperative to assess motivation generally. Many approaches have been developed to assess various aspects of motivation in animals; however, the following section will focus on procedures largely tested in the operant chamber.

**Classical Conditioning: Pavlovian conditioned approach**

One way of examining motivational processes is through assessing Pavlovian conditioned approach. The premise behind this method is that there are stimuli in the environment that promote survival and thus, the stimuli possess natural rewarding properties that are motivating (Robinson et al., 2014). These natural rewards elicit pleasure, act as incentives that motivate behavior, and increase the frequency of the actions that produce the reward, such as food, drugs, sex, etc. (Berridge & Robinson, 2003). While natural rewards activate some proportion of behavior, most of the behaviors that humans perform daily are activated by a previously neutral stimulus that has developed a predictive relationship with a natural reward or biologically significant event or object (Robinson et al., 2014). These previously neutral stimuli that predict the delivery or the availability of a reward are able to acquire several important properties through which they are able to initiate and/or regulate behavior (Robinson et al., 2014). Further,
previously neutral stimuli (i.e. conditioned stimulus) that become associated with a reward can acquire the ability to arouse a conditioned response, which are often similar to the response that is elicited from the reward itself (Robinson et al., 2014). The prototypical example of the conditioned stimulus is through operant conditioning procedures, where animals are trained to interact with some sort of stimulus (i.e., a lever or a button) in order to obtain a reward. In this case, a lever or a button is a neutral stimulus prior to entering the operant chamber; however, after experience in the operant box, the previously neutral stimulus that is associated with the reward acquires the ability to produce a conditioned response, much like the reward itself does from the beginning of training. Hence, the availability of the conditioned stimulus (e.g. the lever or button) results in a response similar to the presence of the reward itself (e.g. food).

Beyond being able to activate conditioned responses, the reward cue may also be able to directly activate motivational states (Bindra, 1978; Lajoie & Bindra, 1976; Rescorla, 1988), which classifies reward cues as acting like incentive stimuli. Incentive stimuli can be defined as stimuli that have three properties due to their relationship with the reward (Cardinal et al., 2002). First, incentive stimuli are sought after, meaning that animals will work to gain access to them. This aspect of incentive stimuli reinforce and motivate reward seeking behaviors over extended periods of time, even if the primary rewarding stimulus is no longer presented. Secondly, incentive stimuli can cause the relevant motivational state, referred to as conditioned motivation, to take over. In essence, this signals to the organism that they can now work for the reward. This causes organisms to seek out the reward immediately or it strengthens seeking behavior. Finally, incentive stimuli solicit attention; if the stimulus can be localized, it will attract an organism, which provokes approach from an organism. This approach response is referred to as Pavlovian conditioned approach behavior (Robinson et al., 2014).
Operant Conditioning: Background

Operant conditioning has been extensively studied for many decades, beginning with B.F. Skinner who is credited for developing the basics of operant conditioning (Staddon & Cerutti, 2003). Skinner postulated that operant conditioning was a form of learning that occurs by using rewards and punishments after the performance of a specific behavior (Skinner, 1938). It is through the association between a certain behavior and a negative and/or positive consequence (e.g., reward or punishment; Skinner, 1938) that an animal learns. Skinner’s original experiments involved pigeons and rats that were trained to peck at a target or press a lever, respectively; upon completing this behavior, the animals were rewarded with food, which is considered to be a naturally rewarding stimulus. These behaviors were conducted in operant conditioning chambers, which allowed for much greater control of the environment, as they are enclosed chambers with only relevant stimuli inside. Based on Skinner’s studies as well as the plethora of operant conditioning studies that have been conducted since, the basic principle of operant conditioning has found largely to be supported (reviewed in: Staddon & Cerutti, 2003; Dalla & Shors, 2009; Bamford et al., 2018). Collectively, research has shown that operant conditioning training increases the likelihood of a target behavior because a stimulus predicts a positive reward or a reduction of a negative consequence.

Operant Conditioning: Schedules of Reinforcement

One important factor in operant behavior is the design of the schedule of reinforcement. Over decades of study, it has become clear that schedules of reinforcement are important to the operant conditioning process (Skinner, 1963) and can impact the strength and rate of responding. Schedules of reinforcement are based on the number of responses and/or the interval of time, and will be discussed in terms of rodent models in operant conditioning chambers with the desired
behavior of a lever press, however the same principles hold for other desired behaviors (e.g. button pushes, screen taps, etc.). The simplest schedule is a fixed-ratio (FR) 1 schedule where a food pellet follows every lever press, also referred to as continuous schedule of reinforcement. Prior research has demonstrated that once rats or other animals learn the association between a lever press and a food pellet on the continuous schedule of reinforcement, they will rapidly press the lever until some presumable point of satiation (Skinner, 1938). Generally, FR1 schedules of reinforcement allow for rapid learning to take place since an animal is reinforced with every single correct response that it makes. Once the reward no longer follows the stimuli, behavior sharply declines, leading to extinction of the behavior. Thus, the continuous schedule produces high rates of responding with a quick reduction in the desired behavior when the reinforcement is omitted.

In addition to the continuous reinforcement schedule, other reinforcement schedules are based on the number of desired behaviors, referred to as ratio schedules. A fixed-ratio (FR) schedule is defined by the number of times that the lever needs to be pressed in order for a reward to be delivered (e.g. FR1 = 1 bar press for each reinforcer, FR5 = 5 bar presses for each reinforcer, etc.). A second type of ratio schedule is the variable-ratio (VR) schedule, where the number of lever presses varies between each reinforcer delivery, but around a specifically chosen average (e.g. reinforcer is delivered after 10 bar presses on average). Both FR and VR schedules generate high rates of responding in rats in Skinner boxes; however, the behavior within a behavioral session can be modified depending upon the type of schedule used. For example, animals will usually pause momentarily after each food delivery when an FR schedule is used but will steadily keep responding in a VR schedule, which can be attributed to the predictive value of food within each schedule (Staddon, 2016). Ultimately, the type of schedule that is used
changes the behavioral outcome. In society, VR schedules have been used in casinos on slot machines because this type of schedule allows behavior to be performed at a higher level once a task is learned. On the other hand, FR schedules are useful in the teaching and sustaining of behavior but would not be as productive in, for instance, slot machines because the behavior would cease once the certain amount of responses had been made. Thus, FR schedules are much more efficient for training and sustainability purposes whereas VR schedules are more useful after learning has taken place.

In addition to response-dependent schedules, schedules of reinforcement can be based on the passage of time, either fixed or variable. A fixed interval (FI) schedule is based on a set amount of time having passed before a reward would be delivered for the targeted behavior (e.g. a rat receives a reward every 30 seconds with the correct response), while a variable interval (VI) schedule is based around an average time having taken place before a reward would be delivered (e.g. every 30 seconds on average a rat will receive a reward with the correct response). Unlike the previously mentioned ratio schedules, interval schedules only require an animal to make one response within the right time interval to receive the reward. In interval schedules of reinforcement, a behavior that is given too soon will not result in a reinforcer because the time interval is required to elapse, such that there is a true “time out” period. In comparison to ratio schedules of reinforcement, interval schedules of reinforcement require longer training periods because the organism is required to associate their response to receive the reinforcer AND a time interval; this is different than both the FR and VR schedules because each of these only requires an animal to learn a response, albeit more responses are needed to receive the reinforcer. Interestingly, FI performance occurs in stages, with an initial learning phase that usually results in accidental behaviors leading to the reinforcer being delivered. Then, in the second stage,
animals start to press at a constant rate followed by a stage where animals begin to accelerate their responding followed by a slowing of responding. Finally, animals show an element of timing their behavior and demonstrate a pause after receiving the reinforcer, which is followed by an accelerating response nearer the time interval to receive the reinforcer (Ferster & Skinner, 1957). This later pattern may be due to the animals eventually learning the timing interval associated with no reward; thus, they reduce their behavior or effort when it does not lead to a positive reinforcer (Staddon, 2016). Likewise, the longer the time interval for the schedule, the longer the pause becomes as well (Staddon, 2016). Interestingly, regardless of the length of the pause, animals seem to underestimate the amount of time that has passed and begin responding again before the full interval has elapsed (Staddon, 2016). Overall, VI schedules of reinforcement produce the most consistent rates of responding due the high probability of receiving the reinforcer associated with responding in a steady manner (Staddon, 2016). Thus, the different schedules of reinforcement allow for more complex operant procedures to be utilized because the behavior that is required to receive reinforcement is nuanced. Although each schedule of reinforcement produces different behavioral outcomes, each is able to provide information on the motivational state of the animal because the task requires effort to receive a reinforcer.

**Operant Conditioning: Theories**

Over decades of research, several principles and theories have been developed to support operant conditioning and to further predict behavior. One well-known principle of operant conditioning is the Premack principle, which explains reinforcement contingencies. The Premack principle predicts the outcome of operant conditioning relationships based on the relative probability of two behaviors connected by a contingency (Knapp, 1976). For operant
conditioning, the Premack principle posits that an animal should be more willing to engage in a less probable activity such as lever pressing if it then has the opportunity to engage in a more probable activity such as eating (Staddon, 2016). Likewise, parents employ the Premack principle with their children by allowing their child who values watching television (high probabilistic behavior) to do so only after completing their chores (low probabilistic behavior). The parents will establish a contingency between the behavior that is less likely to be performed on its own (i.e. chores) with the behavior or activity that one wants to participate in (i.e. watching television). This principle can apply to more than two behaviors and/or activities (Premack, 1965). In one experiment, Premack tested monkeys in individual cages where they were able to play with four different things. After looking at the time spent with each object, Premack set up contingencies so that the item that was played with the least needed to be played with for a certain amount of time before the monkey could gain access to preferred items (Premack, 1965). This principle expanded the conceptualization of reinforcement in operant conditioning to include the option that reinforcement contingency could involve the contingency between two or more behaviors instead of just a stimulus and a behavior (Barton, 2013). Further, it also provided evidence that an organism can be motivated to complete a task in order to gain access to something they want and/or need.

Matching law is a second theory that furthers our understanding of motivational assessment within operant conditioning. Broadly, this law states that an organism will distribute their behavior between alternatives in the same ratio that reinforcement has been obtained from those alternatives, which means that organisms will match their behavior to the value of the reinforcement that is received from each response. Formally, the matching law states that the proportion of the majority of the choices that are made equals the proportion of the majority of
the reinforcements that are obtained (Hernstein, 1961). Hernstein demonstrated this law in the 1960’s in a study of pigeons’ behavior in an operant box. In this study, pigeons were required to peck one of two response keys that were both on a VI schedule in an operant chamber (Hernstein, 1961). Each of the VI schedules was concurrently available and was independent of each other (Hernstein, 1961). The pigeons demonstrated a nearly perfect correlation between the relative rates of behavior against the relative rates of reinforcement, indicating that as reinforcer deliveries increased, behavior was also increased (Hernstein, 1961). Matching law has been instrumental in the explanation of motivational behavior and application for behavioral modification. For instance, if a student is motivated by a teacher’s attention and that teacher provides some attention for good deeds but provides a lot of attention in terms of reprimands, then the student will not be motivated to perform the good behaviors; instead, the student will perform the reprimanded behaviors in order to receive the greater amount of attention (Reed & Kaplan, 2011). While much of the theory that was originally crafted was based on operant conditioning experiments in animals, it contributes to our current understanding of motivated behavior more generally in humans.

When thinking about the theories that further explain operant conditioning as well as the paradigms that have been utilized to assess motivation within an operant conditioning context, one may question whether it is only motivation being assessed in these procedures. Classically, in these procedures, an increase or decrease in behavior was interpreted as a change in learning, rather than motivation. To address the distinction between learning and motivation in animals, motivational theorists test behaviors established through training that have stabilized. In this way, a change upward or downward in behavior following a manipulation suggests an increase or decrease in motivation.
Overall, there are many schedules of reinforcement that can be used to assess motivation. In order to elicit high levels of stable responding, a FR5 schedule of reinforcement is ideal. Prior research has found the FR5 to lead to high levels of lever pressing within a session (Yohn et al., 2016; Yohn et al., 2015; Salamone & Correa, 2002). However, it is important to select an appropriate ratio schedule that sustains behavior at a rigorous level but allows for an experimental manipulation to increase or decrease responding. Previous research from our lab has indicated than an FR5 schedule produces high levels of responding while leaving enough room to manipulate responding experimentally. FR schedules are generally more predictable than other schedules that have been discussed; this predictability allows for the task to be acquired quickly by the animals and for the rats to show stable responding, making it ideal for repeated measures testing.

**Operant Conditioning: Effort-Related Choice Procedures**

Often, there is a separation between an organism and the motivational stimuli that it is seeking, which requires an organism to overcome obstacles or work to obtain the relevant stimuli. This aspect of motivation is referred to as the activational aspect of motivation (Cofer & Appley, 1964). Because an animal is required to overcome obstacles to receive the desirable stimuli they are pursuing, they must allocate resources towards seeking this stimulus, meaning that they must exert effort (Salamone & Correa, 2012). Effort can be assessed through measures of the rate, persistence, and levels of behavior. This effort exertion may be brief at times, but there are other circumstances where effort must be sustained to be given access to the reward (Salamone & Correa, 2012); thus, motivation is highly adaptive. Researchers have suggested that the activational aspect of motivation underlies the concept of ‘wanting’, which has been defined as the desire for a particular stimulus that pushes an organism to pursue and/or consume a
Another aspect of motivated behaviors is choice or the directional aspects to behavior, i.e. behavior is either directed toward or away from a specific stimulus (Cofer & Appley 1964; Salamone, 1988). In their natural habitat, organisms are often forced to make choices between multiple options. For instance, a hungry animal may have access to food but it is near a predator. In this case, the animal must make a choice between eating because it is hungry or increasing the likelihood of its survival by running away. Experimentally, this can be manipulated in several ways by offering animals a choice without any change in effort in accessing each reward. For instance, in a free feeding test, animals are offered the choice between consuming the preferred pellets or a standard lab chow with minimal effort required to obtain either source of food (Errante et al., 2021). This choice test is often used to assess changes in food preference and hunger following drug exposure and serve as a control for more complicated choice procedures (Errante et al., 2021). Other published examples of behavioral tests that include a choice with equal effort are 2- or 4-arm mazes with different rewards available in different arms equal distance from the start location, lever pressing in operant boxes with different rewards associated with different levers, or the two-bottle sucrose test (Muscat & Willner, 1989; Moses et al., 1995; Hart et al., 2018; sex differences reviewed in Becker, 2009). Thus, experimental procedures like these allow for directional aspects of behavior to be explored in greater detail.

While directional aspects are what drive behavior towards a specific stimulus, the activational aspects of motivated behavior allow organisms to show vigorous activity in the initiation and maintenance of motivated behavior, leading to persistent work output in their reinforcer-seeking actions (Randall et al., 2014). The combination of directional and activational aspects of motivation allows an organism to put effort into a task that leads to the preferred
reward. Almost all motivational tasks assess activational aspects of motivation by measuring components such as latency to initiation of a behavior, time to receive the reward, etc.; however, many of these tasks only provide one type of reward, such as a palatable food item or water. Therefore, there is no measure of choice or direction, which is an important component to studying behavior.

To address both directional and activational aspects of motivation, effort-related choice tests were developed, which provide two different reinforcers that require different levels of effort. The tasks allow for both activational and directional aspects of motivation to be measured and provide a more realistic choice for the rodents that are put into this task. More specifically, these types of tasks allow for a more ethological approach to motivational decision making, for organisms frequently must make effort-related decisions based on cost-benefit analyses in order to overcome the response costs that are required to procure motivationally relevant stimuli (Salamone & Correa, 2002, 2012). These tasks offer a choice between high effort actions that lead to a highly valued reinforcer versus a low effort option that leads to a less valued reinforcer (Hart et al., 2018; Bryce & Floresco, 2016; Randall et al., 2014; Floresco et al., 2008). Many paradigms have been developed to study effort-related behaviors in terms of making a choice between rewards, including tasks where there are differing effort requirements on different levers, the progressive ratio/chow choice task and the fixed-ratio 5 (FR5)/chow feeding procedure.

Operant procedures can be utilized where differences in effort are measured through differing ratio requirements on two different levers. In these procedures, animals learn that one lever requires a low amount of lever presses for a small reward while the other lever requires more presses but gives a bigger reward. This is often referred to in the literature as effort
discounting (Floresco et al., 2008). In one study that utilized these procedures, rats had the choice between making a single response on a low-reward lever to receive two pellets or making either two, five, ten, or twenty responses on a high-reward lever to obtain four pellets (Floresco et al., 2008). Under baseline conditions, animals showed a preference for the high-reward lever; however, as the effort requirement increased on this lever (e.g. from 2 to 20 lever presses per 4 pellets), animals began to press the low-reward lever more often (Floresco et al., 2008), indicating that eventually the effort requirement could become too high for the size of the reward. In that study, once the effort requirement was greater than FR5, the time it took to complete the ratio required to receive the higher reward increased substantially (Floresco et al., 2008), suggesting again that higher ratio requirements may become too high for the value of the reward. While these procedures address the effort aspect of motivation, the same reward is provided for both levers, which is not a realistic scenario in most real-life motivationally relevant tasks.

Motivational procedures also have been developed that offer animals a choice between different rewards with different effort requirements. Two tasks that vary reward value and effort are the FR5 task and the progressive ratio task. For these two tasks, the choice is similar: the animal must press a lever for a specified number of times to receive a high valued pellet or the animal can eat the lab chow that is freely available in the operant chamber as the low effort option (Nunes et. al., 2013; Randall et. al., 2012). In the progressive ratio task, the lever requirements progresses to the next ratio requirement after every 15 rewards are received (FR1x15, FR2x15, FR3x15…), while in the FR5 task, the lever requirement remains at FR5 for each session. In both tasks, the response allocation between two different options, pellets (high value) or standard rat chow (low value), can be measured overall (Farrar et al., 2007). While both
tasks assess motivation, the progressive ratio task is more complex and difficult to achieve stability in lever pressing performance. Pilot studies from our laboratory show that the FR5 produces consistent responding within the first several weeks of training and allows for consistent performance in repeated testing (Figures 1A and 1B). Thus, while both procedures are valuable to the study of motivation, the FR5 task leads to more stable responding over time in rats.

Taken together, effort-based choice tasks allow both activational and directional aspects of motivation to be examined, which is essential for understanding motivational dysfunction because clinical presentations of this dysfunction often present as issues with effort and choice. Motivational symptoms related to behavioral activation and effort expenditure, such as psychomotor slowing, apathy, anergia, and fatigue, could be symptoms of Major Depressive Disorder (Tylee et al., 1999; Stahl, 2002; Demyttenaere et al., 2005; Salamone et al., 2006; Treadway & Zald, 2011). In addition, the neurotransmitter dopamine, which has been shown to be involved in motivated behavior, has been examined in this paradigm through drug manipulations, which, in combination with behavioral analyses, help to provide a clearer picture in terms of the theoretical understanding of motivation and the clinical significance of motivational dysfunction. Overall, effort-based choice assessments are valuable to the study of motivation, as they set up more real-life scenarios that would be experienced by humans. Specifically, having the choice between different options with varying degrees of effort required to obtain them is something people experience often; thus, the clinical relevance of these procedures is valuable to studying motivation overall.
Dopamine and Motivation

Throughout the literature, the neurotransmitter dopamine is inherently tied to motivation as it contributes to many types of motivated behaviors. The next section will provide a review of dopamine systems and a discussion of how this system relates to motivation.

Dopamine Synthesis and Chemical Transmission

Dopamine synthesis is a multi-step process that is essential to dopamine’s availability within the brain. Synthesis begins with tyrosine, which is an amino acid that is available through the diet, absorbed into the blood, and then crosses the blood brain barrier. Once inside the neuron, tyrosine is hydroxylated by the cytoplasmic enzyme tyrosine hydroxylase to yield L-3,4-dihydroxyphenylalanine (L-dopa). The hydroxylation of tyrosine is the rate-limiting step within dopamine synthesis; hence the availability of tyrosine hydroxylase determines the availability of dopamine. L-dopa is taken up into the presynaptic vesicles and converted into dopamine by the enzyme L-aromatic amino acid decarboxylase, which is also called dopa-decarboxylase (Schatzberg and Nemeroff, 1995).

In the neuron, dopamine is transported in vesicles where it can be protected from degradation. Physiologically, dopamine release is dependent on the concentration of calcium externally, as it is the calcium influx in response to voltage changes that then leads to presynaptic release of dopamine (Brimblecombe et al., 2015). After calcium entry into the neuronal membrane, the vesicular contents are released into the synaptic cleft and dopamine diffuses to stimulate dopamine receptors (refer to receptor section for more detail). Dopamine receptors are located on the dopamine pre-synaptic nerve terminal (i.e., axonal autoreceptors) and also on nondopaminergic post-synaptic targets (Wolf and Roth, 1987). When released and bound to dopamine autoreceptors, dopamine initiates a negative feedback effect on its own synthesis to
reduce further release of dopamine as a way to self-regulate; however, stimulation of
postsynaptic receptors can cause a variety of changes, which can be seen in many studies
utilizing dopamine-receptor binding drugs (Hahn et al., 1982; Carlson, et al., 1987; Rabey et al.,
1981). Importantly, dopamine binds and dissociates from the receptor quickly and is then either
broken down by catechol-O-methyltransferase (COMT), metabolized by monoamine oxidase
(MAO) or repackaged into synaptic vesicles (Schatzberg and Nemeroff, 1995). While COMT is
located outside of the nerve terminal and only breaks down a small amount of extracellular
dopamine, MAO is located inside of the nerve terminal and is primarily responsible for
dopamine metabolism (Schatzberg and Nemeroff, 1995).

Dopamine Receptors

Dopamine receptors can be broadly divided into two dopamine receptor families, with
each having distinct properties. These two families of dopamine receptors belong to a G protein-
coupled receptor superfamily with different neurochemical and behavioral implications. One
class of dopamine receptors is the D1-like receptor subfamily, which includes D1 and D5
receptors. Stimulation of D1/D5 receptors leads to an increase in adenylyl cyclase, which
ultimately leads to an increase in the second messenger cyclic adenosine monophosphate
(cAMP; Feldman, Meyer & Quenzer, 1997). An increase in cAMP is essential to neuronal
function as cAMP regulates processes such as gene expression, proliferation, apoptosis and
exocytosis as well as many physiological responses including immune system responses, cardiac
contractility, and memory formation (Beavo & Brunton, 2002; Zhang et al., 2016). D5 receptors
are located in the substantia nigra-pars compacta, hypothalamus, striatum, cerebral cortex,
nucleus accumbens, and olfactory tubercle (Khan et al., 2000). On the other hand, D1 receptors
are located throughout the brain, with the highest densities seen in the caudate-putamen and
nucleus accumbens (Feldman, Meyer and Quenzer, 1997). D1 receptors are also found in the ventral tegmental area (VTA; Nair-Roberts et al., 2008), substantia nigra (Abercrombie & DeBoer, 1997), and the prefrontal cortex (PFC; Abi-Dargham et al., 2002).

Dopamine receptors show differences in affinity states, with some presenting with low affinity and some presenting with high affinity for dopamine. Affinity refers to the attraction of the ligand to the receptor. In a low affinity state, the ligand has a low attraction to the receptor; in a high affinity state, the ligand has a greater attractive force to the receptor. Differences in affinity states is thought to be due to the interaction between the receptor and G proteins, specifically whether the receptor is currently attached to a complex with a G protein (Birnbaumer, 1990). The receptor is in a high affinity state when it is coupled with a G protein and can easily bind a dopamine ligand, whereas in a low affinity state, the G protein is uncoupled from the binding site and is less likely to bind dopamine. Drugs that act as dopamine antagonists do not differentiate between the two states of the receptor and bind with equal affinity to the binding site. This distinction is important because G protein coupling to the receptor is required for agonists in order to exert their full action on these receptors, while antagonists are equally effective regardless of the state of the receptor (Feldman, Meyer and Quenzer, 1997).

In addition to the D1-like receptor subfamily, there is also a D2-like receptor subfamily, which is made up of D2, D3, and D4 receptors. Interestingly, D2 receptors were first recognized as distinct receptors from the D1 family when it was noted that they had a high affinity for antipsychotic medication, as antipsychotics are more selective for D2 receptors in comparison to D1 receptors (Meltzer, Matsubara, & Lee, 1989; Schwartz et al., 1993). Stimulation of D2 receptors inhibits adenylyl cyclase, leading to a reduction in cAMP; thus, D2 receptors are distinguished from D1 receptors in that they have opposing intracellular effects (Kebabian &
D2 receptors are coupled to a different G protein than the D2 receptor. More specifically, the D2 receptor family couples with G\textsubscript{i} proteins, which is the protein that inhibits adenylyl cyclase, while the D1 receptor family couples with G\textsubscript{s} proteins that activate adenylyl cyclase (Undieh, 2010). D2 receptors serve as autoreceptors for dopamine neurons (Ford, 2014), which is logical considering the inhibitory role these receptors play. Therefore, when bound, D2 receptors inhibit G\textsubscript{i}-coupled ion channels and reduce dopamine cell firing. The highest levels of D2 receptor binding occurs in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra pars compacta and the flomerular layer of the olfactory bulbs (Boyson et al., 1986; Charuchinda et al., 1987; Gehlert & Wamsley, 1985) as well as in the VTA (Ranaldi & Wise, 2001). In summary, although D1 and D2 receptors overlap in the regions that the receptors are located, their opposing intracellular functions help to distinguish each type of receptor from the other.

**Dopamine Pathways and Neural Circuitry**

The monoamines, including dopamine, are anatomically organized in pathways and support critical behavioral functions. The cell bodies of the dopamine-producing neurons originate in smaller regions in the midbrain or hindbrain and their axons project throughout the brain. Although there are several neural dopamine pathways, there are three major pathways relevant to motivation. The **nigrostriatal pathway** has dopamine cell bodies in the substantia nigra and the axons ascend via the medial forebrain bundle and the internal capsule terminating in the caudate and putamen (or striatum in rodents) and the globus pallidus (Feldman, Meyer and Quenzer, 1997). The **mesocortical pathway** is the second pathway, which originates in the ventral tegmental area (VTA) and innervates many brain regions. This pathway projects to many
forebrain regions, including the limbic cortical areas, septum, amygdala, hippocampus, the nucleus of the diagonal band, and anterior olfactory nucleus (Fuxe et al., 1985).

The third and most studied pathway for reward and motivation is the *mesolimbic pathway*. The literature has shown that the mesolimbic pathway is imperative for motivation for both biological and drug rewards, as indicated by the fact that mesolimbic dopamine neurons are activated during motivated situations (Salamone et al., 2015; Baik, 2013; Nestler & Carlezon, 2006). This pathway originates in the VTA and projects to limbic system structures, including the hippocampus, lateral septum, amygdala, and, importantly for the current study, the nucleus accumbens and hypothalamus.

**Dopamine and Motivation**

For over half a century, dopamine and the mesolimbic pathway has been associated with motivation for natural and drug rewards. The VTA plays a major role in motivation due to its connections with the NAcc, as well as its projection to the amygdala and hippocampus, which enables the reward circuit to influence emotion and memory formation (Tirado & Gallagher, 2008). The intact mesolimbic system is essential to reward/motivational processing, as it supports an association between a stimulus (e.g. pleasant experience) and a response. The anatomical distribution of dopamine terminals through the mesolimbic system allows dopaminergic axons to modulate prefrontal, hippocampal, and amygdala inputs to the NAcc (Brady & O’Donnell, 2004). Because the connection between these structures is essential for other structures to be able to perform properly, it is thought that a malfunction in the mesolimbic system can contribute to the pathophysiology of the motivational component of psychiatric disorders like addiction and depression (Berridge & Robinson, 1998; Nestler & Carlezon, 2006).
Evidence shows that dopamine in the NAcc regulates behavioral activation and choice processes (Salamone et al., 1991, 2003, 2005, 2007; Vezina et al., 2002; Kelley et al., 2005; Phillips et al., 2007). Research has suggested that active instrumental behaviors that are produced and supported by conditioned stimuli are highly sensitive to interference with dopamine systems (Salamone et al., 1991; Salamone & Correa, 2012). Studies that have utilized NAcc dopamine depletions and intro-NAcc injections of dopamine antagonists have supported this idea. For instance, one study found that lesions of NAcc dopamine disrupt Pavlovian approach (Parkinson et al., 2002). In this experiment, animals were trained that a previously neutral visual stimulus (e.g. light) led to a biologically significant unconditioned stimulus. Through repeated pairings, conditioned approach behavior emerged directed preferentially towards the conditioned stimulus relative to a control stimulus presented on the same screen (Parkinson et al., 2002). Dopamine depletions with 6-hydroxydopamine of the NAcc at varying time points before or after the association had been made in the study resulted in a severely impaired Pavlovian approach regardless of when the lesion was made (Parkinson et al., 2002), indicating a role for NAcc dopamine in learning and sustaining the directionality of a response. Further, interference with NAcc dopamine has been shown to alter behavioral responsiveness to cues associated with reinforcers. Nicola and colleagues (2005) trained animals in a task where one tone predicted a reward would be delivered after 100% of correct operant responses while another tone predicted that only a subset of correct responses (15%) would be rewarded. After training, animals responded in high rates to the tone that predicted that they would receive a reward 100% of the time with a correct response but they responded less to the tone that did not predict a reward was guaranteed with a correct response. However, when animals received microinjections into the NAcc with a dopamine D1 antagonist, it was found that responses were significantly reduced to
both tones, indicating an inability to discriminate specific stimuli with a high probability reward (Nicola et al., 2005).

Interestingly, although food-reinforced instrumental behavior can be lowered by NAcc dopamine depletion or antagonism, these effects are schedule-dependent with drug manipulations have no effect on performance at low or moderate ratio requirements but increasing the work requirement in an operant conditioning task increases the suppressing effects of NAcc dopamine depletions or antagonism (Aberman & Salamone, 1999; Salamone et al., 2001; Ishiwari et al., 2004). For instance, one study trained rats on four different FR schedules (FR1, FR4, FR16, FR64; Aberman & Salamone, 1999) and then depleted NAcc dopamine with the neurotoxin 6-hydroxydopamine (6-OHDA). Following the lesion, there were no effects on the behavior with the FR1 schedule, however as the ratio or effort became higher (i.e. FR16 and FR64), the deficit emerged (Aberman & Salamone, 1999). This indicates that NAcc dopamine is sensitive to higher effort requirements in operant tasks, but other systems may support low effort responses. The importance of effort was also illustrated in a study that used escalated ratio requirements as high as an FR300 (Salamone et al., 2001). Interestingly, even when the ratio between lever pressing and food delivery was kept constant (i.e., FR50, one pellet every 50 responses; FR100, two pellets every 100 responses, etc.), there was still an effect of accumbens dopamine depletion with increasing ratio requirements (Salamone et al., 2001). Likewise, with similar procedures but lower ratio requirements, another study found that following accumbens dopamine depletions, there was a significant impairment in FR5 lever pressing with no significant effect on FR1 performance (Ishiwari et al., 2004). Taken together, these findings demonstrate the importance of NAcc dopamine in motivational processes related to activational aspects of motivated behavior that is sensitive to effort requirements of the task.
Several studies have been conducted that demonstrate that dopamine antagonists and agonists influence the effort requirement in motivational tasks. For instance, in one such study, male rodents were assessed in an FR5/chow choice feeding task, where animals are trained to either lever press on an FR5 schedule for a sucrose reward or approach and consume freely available lab chow. After training, male rodents were injected with varying doses of haloperidol, which is a D2 receptor antagonist (Cousins et al., 1994). Rats injected with haloperidol reduced their lever pressing for the highly palatable sucrose reward while chow consumption was increased, indicating reduced motivation for the higher effort option when dopamine is manipulated (Cousins et al., 1994). In a similar manner, a D1 antagonist (ecopipam) decreased lever pressing for a sucrose reward while chow consumption increased in male rodents also on the same FR5 schedule (Yohn et al., 2015). Interestingly, dopamine agonists co-administered with ecopipam were able to restore performance to baseline levels (Yohn et al., 2015), further indicating a role for dopamine in effort and motivated behavior. Finally, our lab recently published the only study that has examined this effortful behavior following the dopamine antagonist haloperidol in both males and females in an operant box. In this study, male and female rodents trained in an effort-related decision making task showed reduced lever pressing for a highly palatable sucrose reward following a systemic injection of haloperidol at the two highest doses (Errante et al., 2021). Taken together, although more work needs to be done on sex differences in this behavior, the findings from these studies indicate that mesolimbic dopamine is critical in effort-based motivated behavior.

Sex Differences in Motivation

Sex differences have been documented in many behaviors throughout the literature. For instance, women perform better in learning tasks requiring verbal or semantic rules as well as
tasks that require memories related to personal experiences, while men tend to perform better in tasks where spatial information must be manipulated (Astur et al., 1998; Kimura, 1999; Collins & Kimura, 1997). These sex differences have been attributed to a variety of factors, including genetic, hormonal and social factors (Hamann, 2005; de Frias et al., 2006; Cahil, 2006; Sambeth et al., 2007). Knowledge of sex differences can help to better understand the etiology and treatment of various psychological disorders more effectively. For instance, as was discussed previously, depression, which includes symptoms of motivational dysfunction, is more common among women compared to men (Breslau et al., 2017; Fervaha et al., 2016). Interestingly, research has shown a connection between sex hormones and dopamine (Tansey et al., 1983; Becker, 1990, 1999; Bazzett & Becker, 1994; Pasqualini et al., 1995; Bethea et al., 2002), which is the primary neurotransmitter system involved in motivated behavior and has clear connections to depression. This suggests a possible link between sex hormones and motivation, which, through a better understanding of the sex differences in motivation, could contribute to better treatment of psychological disorders associated with motivational dysfunction.

Sex Hormones and Dopamine

Behavioral sex differences have been observed in motivated behaviors and one major factor to consider is that sex hormones differ between males and females. Due to the reproductive systems, females have higher levels of the gonadal hormones estrogen and progesterone, whereas males have greater circulating levels of testosterone and its metabolites (Catenaccio et al., 2016; McHenry et al., 2014). The pituitary hormones, follicle stimulating hormone and luteinizing hormone, modulate ovarian steroid hormone synthesis and secretion in response to gonadotrophin releasing hormone from the hypothalamus. For the female, estrogen and progesterone fluctuate throughout the lifespan, regulating the reproductive cycle of the
female primate with the follicular stage (high estrogen, low progesterone) and the luteal phase (high progesterone, low estrogen; Abraham et al., 1972). In rodents, this cycle is referred to as the estrous cycle, which lasts for only four days and is characterized by three different phases (estrous, diestrous, proestrous) that are classified based on different cell types (Marcondes et al., 2002). One of the biologically active estrogens in non-pregnant primates and rodents is estradiol, which circulates at higher concentrations and has greater potency compared to other active estrogens (Blaustein, 2008; Gillies & McArthur, 2010). It is one of the major sex hormones in the body and participates in reproduction, brain function, and behaviors (Lan et al., 2018). Males also have circulating levels of estradiol, which is essential for sexual function in males (Chen et al., 2020). Studies have been conducted that supports relatively equal basal levels of estradiol between males and females (Konkle & McCarthy, 2011) with different behavioral effects between the sexes (Pleil et al., 2011; Lan et al., 2018), indicating the importance of estradiol for both sexes.

Gonadal hormones have been shown to mediate several neurotransmitter systems, including the dopamine system. Through animal research in females, it has been shown that estradiol effects dopamine by increasing synthesis, release, and receptor binding. Specifically, one study showed that when estradiol was added to the superfusing fluid in a dialysis experiment in the striatum, there was an immediate increase in dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), which was taken to mean that estradiol affects dopamine synthesis (Pasqualini et al., 1995). Further, other studies have shown that acute administration of estrogen to ovariectomized rats causes an increase in amphetamine-induced striatal dopamine release, as measured by in vivo microdialysis (Becker, 1990; Becker & Rudick, 1999; Castner et al., 1993). Another study showed that, after a single injection of estradiol, there was a significant increase in
striatal D2 dopamine receptor binding in castrated rats (Bazzet & Becker, 1994). While estradiol enhances dopaminergic function, the effects of progesterone are not quite as clear. For instance, some studies have suggested that progesterone exerts opposite effects to estradiol (Fernandez-Ruiz et al., 1990), while other studies have found similar effects of both hormones (Sanchez et al., 2010), with both studies examining dopamine through the dopamine metabolite DOPAC. However, the specific connection between progesterone and dopamine is not quite clear, with some studies showing that there is an effect of progesterone only when primed with estrogen (Petitclerc et al., 1995) and others showing opposite effects (Cabrera et al., 1993). Thus, while prior research clearly shows a relationship between estradiol with dopaminergic function, the relationship between progesterone and dopamine is less clear.

Additional studies have established sex differences in dopamine in brain regions associated with motivation. One approach to measuring dopamine levels is through in vivo microdialysis, which assesses dopamine levels through a semipermeable membrane inserted into specific brain regions. When comparing neutered rats, it was found that adult castrated male rats have significantly higher levels of dopamine in the striatum and the NAcc, compared to adult ovariectomized female rats (Castner et al., 1993; Cummings et al., 2014). However, there are no sex differences in dopamine uptake, indicating that the sex difference observed in extracellular dopamine is most likely due to a sex difference in dopamine release, synthesis, and/or metabolism (Castner et al., 1993).

Studies examining stimulant-induced dopamine release have provided additional information on the difference in extracellular dopamine between male and female rodents. Stimulants release dopamine by working on the transporter or release, depending upon the specific stimulants, and therefore can be used to better understand the mechanics of dopamine
transmission. In one study, the response to cocaine in ovariectomized female rats and castrated male rats (Cummings et al., 2014) was assessed using microdialysis following an acute injection of estradiol. Estradiol enhanced cocaine-induced dopamine in the dorsolateral striatum in ovariectomized females but not in castrated males, indicating that estradiol preferentially enhances the response to cocaine in females through the function of the dopamine transporter (Cummings et al., 2014). These findings demonstrate that the gonadal hormones differentially regulate dopaminergic activity in brain regions critical to motivated behavior in male and female rodents, providing one mechanism through which sex differences influence motivated behavior.

Influence of Sex Hormones on Motivated Behavior

Through the literature, it is clear that a relationship exists between sex hormones and dopamine. Specifically, as was previously discussed, sex hormones like estradiol are able to modulate dopaminergic activity through their impact on dopamine synthesis, release, and receptor binding. Dopamine is essential for the present study, as it is the neurotransmitter that underlies motivated behavior, particularly effort-based motivated behaviors. Importantly for the current study, it is theorized that this sex-specific change in motivated behavior is due to changes in the dopamine system that is altered by sex hormones. Thus, it is essential to review the literature on sex differences in motivated behavior to better understand this phenomenon.

Several researchers have demonstrated that sex differences exist in motivationally relevant behaviors, including operant conditioning procedures. Previous research has found that males have higher contact rates with the lever regardless of whether it is the active or inactive lever (Dalla & Shors, 2009) and generally lever press more during training in operant tasks compared to females (van Haaren et al., 1990). Females perform better in progressive ratio and fixed ratio tasks compared to male rodents (Grimm et al., 2022). Specifically, when both males
and females were tested in progressive ratio and fixed ratio tasks, it was found that females responded at higher rates for sucrose (Grimm et al., 2022). Importantly, this was not due to a sex difference in sweet preference, as a two-bottle sucrose test was also conducted, which found no sex differences (Grimm et al., 2022).

Sex hormones may modulate decision making procedures for female rats and contribute to cost/benefit evaluations (Uban et al., 2012). One study examined the effect of estradiol and selective estrogen receptor agonists on performance in an effort-related decision making task (Uban et al., 2012). Ovariectomized female rats selected the high-reward option significantly more than non-ovariectomized rats and replacement with high doses of estradiol reduced the selection of the high reward option once again (Uban et al., 2012). When the estrogen agonists were administered to ovariectomized rats, there was an increase in the selection of the high-reward option, which suggested that multiple subtypes of estrogen receptors might be involved in this behavior (Uban et al., 2012). These findings demonstrate that estradiol and stimulation of estrogen receptors play a role in cost/benefit decision making in females.

Recent research has pointed to sex differences in other decision-making tasks that offer a choice between activities. In one study, researchers assessed both males and females in a FR5/chow choice task and found that males pressed the lever more than females overall, which the authors interpreted as a baseline difference in behavior between males and females (Presby et al., 2021). In the same study, male and female rodents were offered a choice between running on a wheel or consuming standard laboratory chow and found sex differences in that females chose to run more and consume less food while males showed the opposite pattern of behavior (Presby et al., 2019). These two findings are interesting to compare, as it suggests that performance may be influenced by task parameters and that one must consider these parameters when interpreting
data from motivational tests. Overall, sex differences have been observed in a variety of motivational tasks throughout the literature, perhaps in part due to different circulating gonadal hormones, and are an important consideration in motivational tasks; as such, both male and female rats were tested for the current studies.

Motivational Dysfunction: The Influence of Stress

Stress is present in many situations that humans experience regularly, such as disruptions in domestic life or pressure from work as well as any personal struggles we may face. In addition, stress has long been associated with the etiology and/or exacerbation of various psychological disorders, especially those with motivational dysfunction like depression (Heim et al., 2008; Martin et al., 2009; Nemeroff, 2016). Prior research has shown that major stressful events precede the first depressive episode in approximately 80% of cases (Mazure, 1998). Studies also suggest reward processing is particularly sensitive to stress and exposure to stress may target dysfunction of reward information processing, which are common in depression (Bogdan et al., 2014).

Several researchers have shaped the way we now think about stress, beginning with Walter Bradford Cannon. His initial book described the bodily changes that accompanied exposure to highly emotional situations, intense exercise, hunger, cold, and pain, among other things. He noted that functions that support the body in a resting state were either intensified or halted in order to have enough energy to deal with the stressful event. Through these observations, he posited that organisms have a ‘fight-or-flight’ response, which has come to describe the sympathetic activity of the peripheral nervous system. Cannon also coined the term homeostasis, which he defined as the ability of an organism to maintain their physiological systems in the direction of a dynamic equilibrium (Cannon, 1915).
Building upon Cannon’s seminal book (1915), Hans Selye merged the ideas of the physical response with the psychological impact of stress. While Selye agreed with Cannon’s interpretation that stress can be encountered in life-threatening situations and that the physiological response produced matches the situation, he believed that organisms could learn to adapt the response and resist it (Selye, 1950). Selye’s expansion of the definition of stress allowed for the inclusion of psychologically based disorders. More specifically, if an individual is unable to adapt or resist the stressors that occur in their life, this could lead to serious physiological and/or psychological consequences, such as depression and anxiety, as well as limited ability to deal with subsequent stressors. He also believed that if this inability to adapt became chronic, this could lead to a stage he termed exhaustion (Jackson, 2014; Selye, 1936), where emotions as well as physical and mental health decline and can even lead to death.

The theory of allostasis more recently emerged to provide an explanation of differences in the ability to adapt or resist a stressor. Allostasis involves the adjustments that an organism may make in response to or in preparation for a change in environmental demands (Robertson et al., 2017; McEwen, 2000). For example, during periods of acute stress, immune function is enhanced and certain types of memory are improved (McEwen & Gianaros, 2011). However, there is a tricky balance when considering allostasis; although the ability to adapt is often advantageous, if the situation that caused the change to begin with continues for extended periods of time, the response to this change could eventually become disadvantageous. For instance, glucocorticoids, which are hormonal mediators of stress, can serve a protective or a damaging role depending on the situation. Specifically, in response to stress, glucocorticoids are able to suppress or stimulate certain bodily functions with the goal of self-preservation (Whirledge & Cidlowski, 2010); on the other hand, chronically elevated glucocorticoids have
been implicated in major depression and anxiety disorders (Papadopoulou et al., 2015). They also act in the brain to increase appetite for food and to increase locomotor activity and food seeking behavior (Leibowitz & Hoebel, 1997). Although these responses are useful after a period of activity, they are not useful during periods of inactivity and when activation is not needed. In these situations, glucocorticoids that are elevated chronically can disrupt efficient function of insulin on glucose regulation and lead to elevated insulin, deposition of body fat, and issues with the coronary arteries (McEwen, 2000). Allostasis would be an effective response to higher glucocorticoids because it would theoretically lead to a reduction in the physiological responses to chronic stress and protect the brain and body from long-term damage.

HPA Axis and the Stress Response

A principal component of the physiological stress response that is activated by disruptions to homeostasis and changed with allostasis is the hypothalamic-pituitary-adrenal (HPA) axis. Generally, the body’s response to stress is self-regulating, meaning that all processes in this system have the goal of returning critical bodily functions to a point that lies within a range that is essential for survival (Stephens & Wand, 2012). In order for this to occur, behavioral, endocrinological and physiological components need to work synergistically. The main structures involved in the HPA axis are the paraventricular nucleus (PVN) of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland (Smith & Vale, 2006). These structures work together in healthy organisms to induce a stress response and, subsequently, to terminate the stress response to maintain a homeostatic state.

The PVN is an essential structure to the function of the HPA axis. In response to a stressor, neurons in the PVN are activated, depending on the nature of the stressor itself (Heck & Handa, 2019). Stressors that involve an immediate threat to homeostasis require the rapid
communication of signals to PVN neurons through direct serotonergic or catecholaminergic projections from the brainstem (Handa & Weiser, 2014; Herman & Cullinan, 1997), with these inputs activating other neural systems within the PVN. With all known acute stressors, the response of the PVN is important for anticipation of a homeostatic challenge and transmitting that information to higher brain structures that may need to interpret the information in regard to the significance of the stressor based on instinct and/or prior experiences (Herman et al., 2003).

The activation of the PVN initiates a cascade of hormonal events resulting in a stress response. Specifically, neurons in the PVN of the hypothalamus synthesize three hormones, corticotropin-releasing factor (CRF), arginine vasopressin (AVP; Stephens & Wand, 2012) and oxytocin (Heck & Handa, 2019). CRF is derived from a preprohormone and secreted from PVN neurons into blood vessels of the median eminence that connects the hypothalamus and the pituitary gland. Through the action of CRF, the anterior pituitary gland is stimulated to produce the precursor protein proopiomelanocortin (POMC), which is the prohormone for several pituitary hormones (Stephens and Wand, 2012). CRF stimulates the production and secretion of adrenocorticotropic hormone (ACTH) that in turn circulates throughout the body. In response to ACTH, glucocorticoid synthesis increases in the adrenal glands and glucocorticoids are then released into the blood stream to act at many tissues including the brain. Glucocorticoids are essential, as these molecules act throughout the body and brain to mobilize energy stores, enhance cognition, and increase cardiovascular activity while simultaneously suppressing immune, reproductive, and digestive functions (Sapolsky et al., 2000). In humans, the glucocorticoid that is released is called cortisol while in rodents, it is corticosterone (Stephens & Wand, 2012), both of which are extensively tied to the stress response of the organisms. In the short term, it has been shown that elevation of glucocorticoids is a key component for the
physiological and behavioral changes for the acute stress response; however, persistent
increases in glucocorticoids from chronic stress can have detrimental health effects (Sapolsky et
al., 2000; Holsboer, 2001; de Kloet et al., 2005).

Importantly, once cortisol or corticosterone levels reach a particular threshold, CRF and
ACTH release is diminished to return the body to a normal, unstressed state once again
(Stephens & Wand, 2012). The secretion of CRF and ACTH is controlled by a negative feedback
loop exerted by cortisol/corticoserone’s action on the pituitary gland, PVN and hippocampus.
Cortisol and corticosterone (collectively referred to as CORT) can bind to two different types of
receptors—the mineralocorticoid (MRs) and glucocorticoid receptors (GRs). While CORT binds
more strongly to the MRs under basal conditions (Gomez-Sanchez & Gomez-Sanchez, 2014), in
stressful situations, circulating levels of CORT increase substantially and bind to GRs. This
binding activation initiates greater physiological and behavioral responses to stress as well as
initiates negative feedback of the stress response (Stephens & Ward, 2012). Thus, through a
delicate negative feedback system, normal levels of cortisol can be maintained.

Interestingly, researchers have discovered that there are sex differences in the HPA axis’
response to stress. Females tend to have a heightened response to acute stressors, which is seen
through elevated levels of CORT and ACTH compared to males following exposure to several
different types of stressors, such as restraint stress and footshock (Babb et al., 2013; Iwasaki-
Sekino et al., 2009). Several studies have supported the finding that females have elevated
CORT and ACTH compared to males. One of the original studies to compare male and females
found that female rats had a much greater increase in CORT levels following an acute ether
anesthesia stressor compared to males (Kitay, 1961). Moreover, females injected with ACTH
showed CORT levels that were almost double those seen in males and remained elevated for
significantly longer (Kitay, 1961). Other studies have also supported that there are sex
differences in the stress response after acute stress exposure. In one study, neutered male and
female rodents were treated with testosterone or estradiol, respectively, and then exposed to one
hour of restraint stress along with intact rodents (Kalil et al., 2013). Overall, it was found that
intact females had higher levels of CORT after restraint stress with no other treatment; however,
ovariectomized females showed a decrease in CORT levels after restraint stress compared to
intact females while intact and castrated males showed no change (Kalil et al., 2013).
Interestingly, when treated with estradiol, females showed a partial reversal of the attenuation in
CORT produced from the ovariectomy while males showed no change with testosterone
treatment (Kalil et al., 2013). Thus, the females have a heightened baseline and stress-induced
CORT response.

It is thought that the individual differences in stress responses may be due to sex
differences within the different components of the HPA axis. In the PVN, neuronal activity after
stress exposure is greater in female rats than males (Seale et al., 2004; Viau et al., 2005; Larkin
et al., 2010). Female rats have also been shown to have greater AVP and CRF mRNA expression
in the PVN and greater expression of POMC mRNA in the anterior pituitary after acute stressors
when compared to males (Viau et al., 2005; Babb et al., 2013; Seale et al., 2004). Researchers
have also shown that female rats have a slower return to baseline in ACTH and CORT levels
after exposure to acute stressors, demonstrating a sex differences in the negative feedback loop
in the HPA axis (Kitay, 1961; Babb et al., 2013; Iwasaki-Sekino et al., 2009). It is thought that
this may be due to the role of estrogen in females. Specifically, studies have shown that estrogen
prolongs ACTH secretion (Burgess & Handa, 1992), which suggests that estrogen may be
impairing glucocorticoid receptor negative feedback (Handa et al., 1994). To further support this
theory, researchers have shown that there are alterations in the ability of glucocorticoids to regulate hormone secretion when estrogen is present, with estrogen leading to higher glucocorticoid levels (Burgess & Handa, 1992). Thus, female rats seem to display a heightened response to stress and an attenuated sensitivity in the regulation of the stress circuitry through negative feedback loops compared to males.

Corticotropin-Releasing Factor (CRF)

CRF has come to be regarded as a key regulator of the HPA axis. It is a 41-amino acid neuropeptide that is conserved across species (Vale et al., 1981). CRF acts both as a hormone and a neurotransmitter messenger depending upon the site of release. CRF is released from neurons in the PVN and is the primary activator of the HPA axis (Rivier & Vale, 1983; Vale et al., 1981). In this case, CRF acts as a hormone, where it can stimulate the stress response through its actions on the HPA axis (Schreiber & Gilpin, 2018). CRF is also able to act as a neuromodulator or neurotransmitter at pre- and postsynaptic sites (Lowry & Moore, 2006). CRF enhances or attenuates neuronal activity in one of two ways: by interfering with the activity of the ion channels or by increasing or decreasing the activity of neurotransmitters by directly acting on its own receptors (van den Pol, 2012). CRF is expressed widely throughout the brain, which allows it to modulate the activity of many neuronal systems (Vandael & Gounko, 2019). Because it functions as both a neuromodulator and a hormone, it can be released in multiple different areas and can influence activity over a wider area. Without this functionality and distribution of CRF, the central and peripheral stress response would be drastically different.

Two types of CRF receptors have been identified within the mammalian CNS, which are CRF type-1 receptor (CRFR1) and CRF type-2 receptor (CRFR2) (Schreiber & Gilpin, 2018). Although both receptors are G protein coupled receptors, CRFR1 binds CRF with higher affinity
than CRFR2, while CRFR2 has a much higher binding affinity for other peptides associated with CRF rather than for CRF itself (Tellam et al., 2002; Hauger et al., 2006). Once either receptor is bound, CRFRs signal through G protein coupling, which result in adenylate cyclase activation (Bonfiglio et al., 2011). The increase in adenylate cyclase leads to the generation of the second messenger cAMP, which activates protein kinase-A (PKA; Bonfiglio et al., 2011). Because of the phosphorylation of PKA, binding of CRFRs can then induce gene transcription (Bonfiglio et al., 2011) and influence cellular processes.

The two CRF receptors are quite different in many ways, particularly in terms of expression location and behavioral outcomes. More specifically, CRFR1 receptors are widely expressed in the brain with high concentrations in several regions including the cortex, cerebellum, hippocampus, globus pallidus, and the VTA (Chalmers et al., 1995; Henckens et al., 2017; Van Pett et al., 2000). As was previously mentioned, CRFR1 binds to CRF with high affinity (Bittencourt et al., 1999) and its receptor expression in the brain aligns with areas where the peptide CRF itself is expressed in high concentrations (Schreiber & Gilpin, 2018). In terms of behavior, CRFR1 has been thought to be pro-stress because increases in CRFR1 signaling are anxiogenic (Dunn & Berridge, 1990), while antagonizing or knocking out CRFR1 receptors reduces anxiety behaviors (Henckens et al., 2017; Muller et al., 2003; Timpl et al., 1998; Zorrilla et al., 2002). On the other hand, CRFR2 receptor expression location is more restricted, as it is limited to subcortical brain regions such as the amygdala and lateral septum (Chalmers et al., 1995; Van Pett et al., 2000). While CRF has a low affinity for CRFR2 compared to CRFR1, CRFR2 has high affinity for other endogenous ligands such as the urocortins (Ucns; Schreiber & Gilpin, 2018). Because of this, regions that have high CRFR2 expression also have high urocortin expression and lower CRF expression (Schreiber & Gilpin, 2018). Behaviorally, the
CRFR2 receptor is thought to be important in the recovery from an acute stressor and maintaining homeostasis, which may be due to its contribution to negative feedback (Hillhouse & Grammatopoulos, 2006; Henckens et al., 2017); however, less is known about the CRF2 receptor compared to the CRF1 receptor. Although they appear to serve different roles in the stress system, both CRFR subtypes are important to the functionality of CRF.

While the CRF system plays a central role in the stress response, it is also active in other areas of the brain that are not associated with stress. CRF plays an important role in regulating the mesolimbic dopamine system and motivated behaviors. CRF neurons project from the limbic forebrain and the PVN into the VTA, a primary structure in the mesolimbic dopamine system (Rodaros et al., 2007). Labeling studies have shown that the vast majority of VTA neurons that express CRFR1s are dopaminergic, suggesting that CRF has a key role in regulating mesolimbic dopamine output (Van Pett et al., 2000; Wang & Morales, 2008). Through the use of extracellular recordings, it has been found that CRF increases the firing rate of VTA dopamine neurons through CRFR1 as the CRFR1 antagonist CP-154,526 blocked the effects of CRF (Korotkova et al., 2006; Wanat et al., 2008). However, both CRF receptor subtypes are expressed in the VTA and a more recent study has indicated that CRF can modulate dopamine neurons through activating both receptor subtypes (Haass-Koffler & Bartlett, 2012). It is also thought that CRF may be responsible for modulating excitatory and inhibitory inputs to the VTA since it receives inputs from CRF-glutamatergic and CRF-GABAergic neurons (Tagliaferro & Morales, 2008). Specifically, while it was found that both glutamatergic and GABAergic neurons were involved in the modulation of CRF inputs, the glutamatergic neurons are especially important due to the fact that most synaptic interactions between CRF and dopamine are asymmetric (Tagliaferro & Morales, 2008), which are excitatory. This arrangement suggests that
glutamatergic neurons with CRF immunoreactivity may be part of the circuitry that mediates the stress response within the mesolimbic dopamine system (Tagliaferro & Morales, 2008).

Further, the excitatory and inhibitory actions of CRF on dopamine neurons within the VTA can lead to other major neurological changes. More specifically, studies have shown that the excitatory effect of CRF involves fast events, such as NMDA receptor-mediated synaptic transmission, while the inhibitory effects involve slow forms of synaptic transmission, resulting in long-term plasticity (Beckstead et al., 2009). The NMDA receptor activation is particularly important for these processes, as it is required for long-term potentiation in VTA dopaminergic neurons; thus, it is thought that CRF receptor activation may modulate longer-lasting changes in plasticity (Bonci & Malenka, 1999; Ungless et al., 2001; Bonci & Borgland, 2009). Interestingly, the CRFR2 requires the CRF binding protein, which has been suggested to mediate longer-lasting changes in plasticity (Bonci & Malenka, 1999; Ungless et al., 2003). Thus, CRF is able to modulate dopamine neuron activity in the VTA, which, because of the involvement of the VTA in the mesolimbic dopamine system, can lead to long-term influences on motivated behavior.

Sex Differences in CRF

As the central stress neuropeptide, CRF is thought to be a key contributor to a variety of psychological disorders, including major depression and posttraumatic stress disorder (Austin et al., 2003; Bremner et al., 1997; De Bellis et al., 1993). These disorders show a greater prevalence rate in women compared to men (Breslau, 2002; Kessler et al., 2012) and because of this, researchers have begun to examine sex differences in CRF in rodent models to try to better understand female sensitivity to these disorders (reviewed in Bangasser, 2013).

Sex differences in CRF receptor binding and function have been found in different areas of the brain associated with stress. While broad studies on this topic have not been conducted,
sex differences have been shown in several regions of the brain, including the NAcc, the locus coeruleus, the cortex, and the amygdala. Females show greater binding compared to males in CRFR1 receptors in the NAcc, as well as in certain regions of the amygdala (Weathington & Cooke, 2012; Weathington et al., 2014); however, this topic is still being researched so that a clear understanding is possible. Additional studies have found that female rats have increased coupling of the CRFR1 to Gs binding protein, leading to greater signaling in the cAMP to PKA pathway, in the locus coeruleus (Bangasser et al., 2010). The locus coeruleus-arousal system, which houses the majority of noradrenergic neurons, is modulated by CRF with CRF or CRF agonists increasing the activity of norepinephrine neurons, also active during acute stress conditions (Dunn & Swiergiel, 2008; Plotsky et al., 1987). Moreover, the magnitude of locus coeruleus activation elicited by stress was significantly higher in females compared to males, regardless of hormonal status, indicating a potential sensitivity of locus coeruleus neurons to CRF (Curtis et al., 2006). Importantly, CRF was more potent in activating locus coeruleus neurons in female rats compared to male rats (Curtis et al., 2006). Interestingly, this effect was seen in all female groups regardless of hormonal status compared to the male groups (Curtis et al., 2006). Taken together, these studies demonstrate that there are sex differences in the effects of stimulating CRF receptors; however, whether these sex differences in CRF contribute to behavioral differences in response to stress between males and females is not known.

Because of the region-specific differences that have been seen with CRF receptors, researchers have begun to speculate that the neural circuitry that responds to stress is also likely to be influenced by sex. In one study that examined this circuitry, CRF was centrally infused into the lateral ventricle and animals were subsequently assessed for neural activation in several brain regions (Wiersielis et al., 2016). CRF administration activated many brain regions that are
known to be critical for stress and motivation, including the NAcc and the PVN. Interestingly, hormonal stage of the female rat impacted the CRF-induced cFOS (marker for neuronal activity) activation in the dorsomedial periaqueductal gray, laterodorsal tegmental nucleus, ventromedial dorsal raphe, basal nucleus of Meynert, and NAcc shell. CRF injection increased cFOS only in diestrous females in the dorsomedial periaqueductal gray, laterodorsal tegmental nucleus, and ventromedial dorsal raphe while cFOS was increased in proestrous females and males in the basal nucleus of Meynert and NAcc shell when compared to animals that had a vehicle injection (Wiersielis et al., 2016). There were significant differences between males and females in the NAcc shell, NAcc core, and PVN. Further, proestrous females had a greater correlation for cFOS neuronal activation between brain regions compared to the other groups tested (males, diestrous females). Proestrous females had the greatest differences compared to males in CRF-induced neuronal activation (Wiersielis et al., 2016). Thus, hormones and CRF interact to influence neuronal activation, which may explain the sex differences that are present in stress.

Stress, CRF and Motivated Behavior

Given the central role of CRF in stress responsiveness and its ability to modulate the mesolimbic dopamine system, research has begun to investigate the effect of stress and CRF in motivated behavior through the mesolimbic dopamine system. Holly and colleagues (2016) examined the relationship between CRF and VTA dopamine after social defeat stress and subsequently examined cocaine self-administration to see how these manipulations influenced drug motivation. Through in vivo microdialysis of CRF in the VTA during and after social defeat stress, it was found that CRF is phasically released in the posterior VTA during acute defeat; however, during repeated defeat, CRF is then recruited into the anterior VTA. The researchers then examined the impact of intra-VTA antagonism with both CRFR1 and CRFR2 antagonists
(CP376395 and Astressin2B, respectively) during each social defeat, which prevented the escalation of cocaine self-administration. Importantly, the increase in CRF in the VTA following social defeat stress influenced the motivated behavior in stressed animals long after the stressor had ceased, with stressed rats showing significant cocaine seeking even after 15 days of abstinence while non-stressed controls were not exhibiting this behavior. Importantly, previously stressed rats continued to have elevated CRF levels within the VTA, with antagonism reversing cocaine seeking. Taken together, findings indicate that there is a clear relationship between dopamine and CRF that shows itself in the mesolimbic system and through motivated behavior.

Acute stress alone can impact motivated behavior. Cost/benefit decision making, such as the previously mentioned effort-related choice, is thought of as a task that could be sensitive to an acute stress manipulation. Pioneering work in this area indicated that decision-making is impaired during conditions of acute stress, which causes an organism to rely on more habitual behavior instead of making a more highly valued or preferred choice (Elliott & Packard, 2008). In one study, rodents were exposed to restraint stress for one hour prior to being tested in an effort-related decision-making task that required lever pressing for either option (Shafiei et al., 2012). Rats exposed to restraint stress had a reduced selection for the higher effort choice and had longer choice latencies (Shafiei et al., 2012). Further, the study also found that dopamine activation may contribute to the increased latencies that are caused by stress, which was seen when treatment with a D1 dopamine antagonist before restraint stress attenuated the effects on latency that were previously seen (Shafiei et al., 2012). Thus, acute stress can impact the function of dopamine and, through altering dopamine, is able to reduce motivated behavior.

Similar to exposure to behavioral stressors, several studies have been conducted that demonstrate an influence on motivational behavior when interfering with the connection between
CRF and dopamine. Most of these studies have used CRF antagonists in combination with stressors to directly compare the influence of CRF on dopamine neurons in the VTA as well as the projections form the VTA to the NAcc. Wanat and colleagues (2013) utilized a progressive ratio task in order to examine whether CRF antagonists blocked the effect of acute stress on motivation to work for food rewards. Male rats received bilateral intra-VTA injections of alpha-helical CRF, a CRF receptor antagonist, or vehicle control and then underwent 20 minutes of acute restraint stress before being put into the progressive ratio task. Following exposure to restraint stress alone, the breakpoint was reduced compared to baseline performance but the CRF antagonist microinjected into the VTA returned the performance to baseline levels. Moreover, bilateral injections of exogenous CRF into the VTA reduced breakpoint in a similar manner as stress alone. Utilizing fast-scan cyclic voltammetry during the progressive ratio sessions, the authors further showed that CRF infusion in the VTA produces a selective inhibition of dopamine release to reward delivery without impacting dopamine release to reward-predictive cues (Wanat et al., 2013). A more recent study examined the role of CRF in dopamine-dependent behavior as measured in an effort-related decision making task (Bryce & Floresco, 2016). Specifically, male rats were exposed to one hour of restraint stress, which reduced preference for the high effort, high reward option; however, this effect was attenuated by infusions of the CRF antagonist alpha-helical CRF (Bryce & Floresco, 2016). Interestingly, infusions of CRF into the VTA mimicked the effect of restraint stress on decision making and increased decision latencies (Bryce & Floresco, 2016). Taken together, these findings indicate a clear relationship between CRF, dopamine, and motivation, suggesting that the CRF in the VTA is able to negatively alter dopamine levels, leading to changes in motivated behavior.
CHAPTER TWO: CURRENT EXPERIMENT

Previous research has indicated that stress can influence motivation, with organisms displaying motivational dysfunction after stress exposure (Willner, 2005; Gourley et al., 2008, 2009; Taslimi et al., 2019). Research has shown that female rodents tend to exhibit a heightened response to stress exposure, as indicated by elevated levels of stress hormones (Babb et al., 2013; Iwasaki-Sekino et al., 2009). Preliminary findings from our own research have shown that a pharmacological stressor is able to produce a motivational deficit in both male and female rodents. Further, studies have shown that dopamine and CRF interact to produce deficits in the effort-related choice (Bryce & Floresco, 2016). Despite previous research investigating the influence of stress on motivated behaviors as well as the interaction between stress and dopamine, no studies have examined the relationship between these two systems as a critical mechanism to account for observed sex differences in motivation. Further, no studies have tested the effect of stress on a motivational test that provides different rewards depending upon effort. Therefore, the current project examined whether acute stress altered effort-related motivated behavior (Experiment 1); whether inhibiting selective components of the stress HPA axis attenuated stress-induced behavioral motivational deficits (Experiment 2); and whether acute stress or CRF altered dopamine in the mesolimbic system (Experiment 3).

Previous research from our laboratory has shown that there are sex differences in motivated behavior. In a progressive ratio task where the ratio increases on a logarithmic scale, our laboratory has shown that female rats work harder and are more motivated for the banana
pellet compared to male rats. On the other hand, in the PROG/chow choice task under conditions of food restriction or no food restriction, food restricted males exerted more effort for the high effort/high reward option compared to food restricted females and non-restricted animals of both sexes (Errante et al., 2021). However, in this later task, the sex differences were not present in non-food restricted animals or following the dopaminergic D2 receptor antagonist, which reduced performance for a food reward that required effort but not for free available laboratory chow, suggesting that dopamine may be the final output influencing effort-related motivated behavior for both sexes.

Based on previous studies in the laboratory that indicated sex differences in motivational tasks under increasing ratio requirements, it was of interest for the current studies to find a choice task that did not result in pre-manipulation sex differences so that the effect of stress could be observed in both sexes when starting from the same behavioral point. In pilot data for the project utilizing the FR5 choice task, we found that there were no sex differences in training performance (Figures 1A and 1B). Using the FR5 task, both males and females perform the task to an equal level at baseline as measured by lever pressing and chow consumption. Choice tasks are also more realistic to what an animal may encounter in its natural environment compared to procedures involving a single reward/effort requirement. Thus, the FR5 choice procedure was used due to the equal task performance of males and females prior to stress exposure and to provide measurements of food choice and effort separately. This task provides further information that can inform treatments targeting clinical motivational dysfunction.
Figure 1: Pilot data from the FR5/chow choice task showing baseline performance compared to performance with the pharmacological stressor, yohimbine (2.0 mg/kg). Results indicate no significant sex differences; however, there is a significant effect of yohimbine on lever pressing (Figure 1A) performance in both males and females. Importantly, chow (Figure 1B) did not change, which is standard within this task.
Experiment 1

Experiment 1 - Rationale

Previous studies have indicated that acute stressors and stress-related hormones can influence motivated behaviors and lead to motivational dysfunction (Krishnan & Nestler, 2011; Logrip et al., 2012). However, little is known about the effect of acute stressors on effort-related decision-making procedures. Thus, to determine whether acute stress alters performance in an effort-related decision making task, Experiment 1 exposed rodents to two types of acute stressors and then tested motivated and effortful performance in the choice task. Pilot data from our laboratory has shown that acute exposure to the pharmacological stressor yohimbine reduces lever pressing significantly in both male and female rodents in the choice task (Figures 1A and 1B). Previous research has also indicated that restraint stress is able to reduce selection of high-effort/high reward options when a lower-effort option is also available (Shafiei et al., 2012). To further explore the effects of acute stress on effort-related motivational tasks, two types of stressors were compared—restraint and yohimbine exposure on motivated behavior in both male and female rodents. Both of these stressors activate glucocorticoid release and the HPA axis (Braun & Hauber, 2013; Seki et al., 2018; Leal & Moreira, 1997) but through different mechanisms that may have differential effects on motivational behaviors. Specifically, restraint stress increase directly ACTH and CORT levels in animals (Buynitsky & Mostofsky, 2009), while yohimbine acts by increasing norepinephrine, which plays an indirect excitatory role in regards to HPA axis activation (Plotsky et al., 1987); thus, yohimbine is able to increase stress through its effect on norepinephrine and activation of the sympathetic nervous system and HPA axis.
Experiment 1 - Hypotheses

Based on evidence from previous studies as well as preliminary data from the laboratory, I hypothesized that acute stress exposure would decrease motivated performance in an effort-related decision making task for the high valued food reward. I predicted that this effect would be most pronounced in females, as research has indicated that females are more sensitive to the effects of stress (Viau et al., 2005; Handa et al., 1994; Babb et al., 2013). I also predicted that the effect of stress would depend upon the specific stressor due to their differing mechanisms of actions, with yohimbine producing the greatest decrease in high effort choices and restraint stress reducing high effort choices to a lesser extent but still greater than baseline.

Experiment 2

Experiment 2 – Rationale

Experiment 1 examined whether acute stressors reduce effortful motivated behavior, which would support prior research that stress can increase hypomotivated behaviors (Willner, 2005; Taslimi et al., 2019). However, the neurocircuitry underlying these differences must be better understood to effectively treat both males and females experiencing disorders of motivation. Previous studies have shown that CRF infusions influence motivated behavior (Bryce & Floresco, 2016), but whether CRF alters effort-related choice behavior has yet to be investigated in male and female rats; thus, Experiment 2 investigated the relationship between CRF and effort-related behaviors in both sexes. Research has shown that CRF is able to reduce the excitability in the VTA, which is an essential component of the dopamine circuitry underlying motivated behavior in both males and females (Errante et al., 2021; Douma & de Kloet, 2020). Therefore, Experiment 2 tested whether systemic CRF administration alters...
motivated choice behavior similar to acute stressors and whether pretreatment with a CRF antagonist attenuated restraint-induced changes in motivated choice.

Experiment 2 – Hypotheses

Based on previous studies, I hypothesized that CRF would decrease effort for a palatable food reward as a measure of less effortful/hypomotivated behavior (Yohn et al., 2016; Randall et al., 2012; Bryce & Floresco, 2016). I predicted that this effect would be more pronounced in females based on previous data from the lab (Errante et al., 2021). Further, I predicted that administration of the CRF antagonist prior to restraint stress would attenuate the predicted decrease in effort for the palatable food reward but not for standard rat chow based on previous studies (Bryce & Floresco, 2016). Additionally, it is predicted that the effect of restraint stress would be more pronounced in females but that administration of the CRF antagonist would bring both males and females back to baseline levels of performance.

Experiment 3

Experiment 3 – Rationale

In psychological disorders like major depressive disorder, stress responses are maladaptive (Segal et al., 1992). Outside stressors and stress hormones have been shown to contribute to depressive episodes in humans and depressive-like behaviors in rodents, including reducing motivated behaviors (Hammen, 2005; Sinha et al., 2018). The mesolimbic dopamine system is essential to motivated behavior (Salamone & Correa, 2012) and, importantly for the current study, this system is sensitive to stress (Baik, 2020). Within the mesolimbic dopamine system, the nucleus accumbens is especially important for motivation and depressive behaviors under conditions of acute stress (Salamone & Correa, 2012; Baik, 2020). There are changes in nucleus accumbens dopamine in response to various types of acute stress exposure (Abercrombie
et al., 1989; Lillrank et al., 1999; Puglisi-Allegra et al., 1991; Chen et al., 2015; Cui et al., 2020). Moreover, the stress peptide hormone corticotropin-releasing factor (CRF) influences the activity of the mesolimbic pathway and motivation (Wanat et al., 2013), indicating a further connection between stress and motivation; however, this connection is not well-established and further research needs to be conducted to understand the relationship between CRF, motivation and dopamine. To the overarching hypothesis that acute stress leads to changes in CRF that can alter mesolimbic dopamine and motivated behavior, Experiment 3 measured dopamine in the mesolimbic terminal region, the nucleus accumbens, after stress manipulations.

**Experiment 3 – Hypotheses**

Based on previous studies, I predicted an increase in dopamine in the nucleus accumbens after exposure to the stressors compared to baseline animals and animals injected with saline. While no studies have examined sex differences using similar procedures, I predicted that dopamine would be elevated to a greater extent in females compared to males, as previous studies indicate that females are more sensitive to stress (Babb et al., 2013; Iwasaki-Sekino et al., 2009). While I expected an overall elevation, I expected that this might be stressor specific, as preliminary data indicated that some stressors are more intense compared to others.

The results of these studies collectively determined the role of acute stress on effort-related choice behavior (Experiment 1), whether CRF was able to affect effort-related decision making (Experiment 2), and whether dopamine levels in the mesolimbic terminal region were altered by stress exposure, which may contribute to changes in motivated behavior (Experiment 3). Additionally, the current experiments examined whether there are sex differences in stress-induced changes in this effort-related decision-making task to determine whether stress responsiveness accounts for the sex differences in the behavior through stress-induced changes in
the mesolimbic system. Together, these findings have important implications for disorders characterized by hypomotivation, particularly where sex differences are apparent, such as depression and anxiety.
CHAPTER THREE: METHODS

Animals

A total of 106 (53 males, 53 females) adult Sprague-Dawley (Harlan derived) rats from the Psychology Department’s animal colony at Northern Illinois University were used in the study. Lights were maintained on a 12-hour light/dark cycle (lights on at 6:00am) with temperature maintained at 22±2° C. Food and water were provided ad libitum. Although previous research has shown that food restriction is able to increase responding for a sucrose pellet in certain procedures (Errante et al., 2021; Yohn et al., 2016; Randall et al., 2012; Nunes et al., 2013), chronic food restriction was not used because preliminary data indicated that it was not necessary and it is used as a chronic stressor (i.e., chronic mild stress; chronic unpredictable stress; Marinelli et al., 1996; Sedki et al., 2013). The procedures for the current studies were approved by the local Institutional Animal Care and Use Committee and followed the Guide for the Care and Use of Laboratory animals (National Research Council, 2011).

In Experiment 1, all animals received all treatments (n = 48, 24 females and 24 males). In Experiment 2, all animals received all injections/stressor combinations (n = 36, 18 females and 18 males). In Experiment 3 (n = 22, 11 females and 11 males), a between subjects design was utilized so that each animal received one of the treatments. Group sizes for all experiments were calculated using G*Power (Version 3.1.9.2, Universitat Kiel, Germany). For all experiments, an effect size of 0.8 with an alpha error probability of 0.05 and power of 1.0 indicated a total sample size for the proposed studies should be 72 animals when added together from separate analyses (24 animals required for each project). However, due to individual variability within the
behavioral task and potential other technical difficulties, the additional animals were added into Experiments 1 and 2 to account for the different physical and pharmacological stressor

**Drugs**

Yohimbine HCl (Sigma, St. Louis, MO, USA) was dissolved in sterile water and administered at a dose of 2 mg/kg to male and female rodents in Experiment 1 (Chen et al., 2015). In Experiment 2, the CRF antagonist antalarmin HCl (Tocris, Minneapolis, MN, USA) was systemically injected intraperitoneal at a dose of 10 mg/kg (Furman, 2017; Zorrilla et al., 2002; Ducottet et al., 2003). It was dissolved in 0.5 mL of ethanol (100%) to create stock solution and then diluted to its final concentration with sterile water. For Experiments 2 and 3, CRF (Sigma-Aldrich) was systemically administered at a dose of 1 ug/kg (Lovelock & Deak, 2020) and was diluted in sterile water.

**Assessment of Estrous Cycle**

Beginning one week prior to the start of drug testing and continuing every day through the end of drug testing, vaginal smears were performed daily on all female rats each morning for estrous cycle tracking. One hundred µl of saline (0.9%) was gently pushed into the vagina with an eyedropper and then drawn back, placed on a slide, and viewed under a microscope at 10x power (Becker, et al., 2005). Diestrus was classified when the sample had a dominance of leukocytes and larger cells that were not nucleated. Proestrus was classified when the sample had a dominance of nucleated cells, and estrus was classified when the sample had a dominance of cornified cells (Becker, et al., 2005).
Behavioral Testing

Sucrose Habituation

To habituate rats to the banana flavored sucrose pellets (BioServ Product #: F0059), 50 banana pellets (45 mg) were placed into the home cage of the pair-housed rats for 1 day.

Autoshaping

All rats in Experiments 1 and 2 began their exposure to the lever and operant box with an autoshaping procedure (Berridge & Robinson, 2003). We found in our laboratory that this procedure leads to faster training in subsequent procedures (unpublished data). In each test session, an illuminated lever was introduced into the chamber for 8 seconds. A banana pellet was then delivered at a variable interval (VI) on a 90 second schedule after the removal of the lever. The rat was not required to respond to receive the banana pellet, but associates the delivery of the reward with the signal (i.e. illuminated lever). The autoshaping sessions consists of 25 trials for 5 consecutive days.

Training

Rats in Experiments 1 and 2 were trained in the FR5/chow choice procedure following autoshaping training. The FR5/chow choice procedure offers animals a choice between a high effort/high reward option versus a low effort/low reward option (Yohn et al., 2016; Yohn et al., 2015; Salamone et al., 2002). For 1 week, rats were trained on a fixed interval schedule that provides a banana pellet for every 5 lever presses (FR5) (high effort; 30 minutes maximum). The lever stayed continuously active during these sessions. For the next two weeks, the rats were presented a choice of pressing the lever on the FR5 schedule for a banana pellet (high reward)
and/or consuming the lab chow (low reward) with a similar nutritional make up to the banana pellets that was placed on the floor of the operant chamber (low effort). The rats were freely able to consume lab chow throughout the 30 minute testing session. Chow intake was measured for every training session by weighing the food before the session and then weighing the remaining food (including spillage) after the session. These procedures continued for two weeks (5 days per week) or until stability is reached, which was determined by calculating 25% difference scores for each animal. All animals lever pressed for the majority of the testing session with minimal lab chow consumption, as seen in previous studies (Nunes et al., 2013; pilot data).

Experiment 1

Figure 2: Timeline of Experiment 1 procedures.

Following training, each rat was tested in 3 conditions: 2.0 mg/kg dose IP injection of yohimbine HCl, sterile water IP injection, or restraint for 60 minutes prior to testing. Each condition was counterbalanced between groups. For a yohimbine or water injection test day, animals were injected, returned to their home cage, and placed into the operant boxes 15 minutes later. For restraint, rats were placed into a restrainer for 60 minutes total, removed and allowed to groom themselves for 10 minutes in their home cage so that they do not do this in the operant box during testing, and then placed into the operant boxes. After all animals were tested with
each of the acute stressors, half of the rats received a final yohimbine injection and the other half were put into restraint stress once again to conduct free feeding procedures to assess the effect of yohimbine on food preference and hunger (described below).

**Acute Stressors**

For restraint stress, animals were placed in a standard restrainer and were confined to the restrainer (Harvard Apparatus) for 60 minutes, where the rat was unable to move extensively or escape the restrainer. Yohimbine HCl and water injections took place 15 minutes prior to testing. All stressors were spaced out by a minimum of 5 days to reduce the risk of cumulative effects of the stressors.

**Experiment 2**

![Figure 3: Timeline of Experiment 2 procedures.](image)

Animals in Experiment 2 followed the same training procedures as Experiment 1, training in autoshaping, then FR5 for one week and finally one week or until stable responding with a choice of lab chow or banana pellets. Following the last training day, all animals were tested in a counterbalanced order, with a washout period of approximately 5 days between tests:
1) vehicle + restraint, 2) antalarmin + restraint, or 3) CRF. Animals receiving antalarmin or vehicle in combination with restraint were injected 80 minutes prior to testing, after which they were placed into restraint for an additional 60 minutes for a total of 140 minutes prior to being placed into the operant chamber. All animals in either restraint condition were returned to their home cage for 10 minutes in order to groom before operant testing began. Animals receiving CRF injections were tested 60 minutes after the injection.

Experiment 3

Twenty-two rats were used for this experiment. Three were used for baseline measurements and four received saline injections to control for the injections that were given. The remaining animals were assigned to the remaining conditions as listed in Table 1. Animals were exposed to their respective condition and then rapidly decapitated for brain collection after the lead times for the manipulation had elapsed. The lead times were based on prior studies that showed one hour of restraint stress led to measureable changes in nucleus accumbens dopamine (Imperato et al., 1991; Puglisi-Allegra et al., 1991; Lillrank et al., 1999). Further, a study examining dopamine in the nucleus accumbens after yohimbine exposure found levels to be stable 30 minutes after yohimbine was injected (Chen et al., 2015), indicating that a change may take place sooner than the 30-minute threshold. Based on the literature, it was thought that this timing was sufficient to see changes in dopamine content in response to stress in the nucleus accumbens. For CRF, timing parallels the behavior study in Experiment 2, as this was the lead time used in previous work that produced a behavioral deficit (Lovelock & Deak, 2020). After extraction, each brain was promptly frozen on dry ice.
Table 1: Table of stressor type and lead times for Experiment 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample Size</th>
<th>Lead Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-manipulated control</td>
<td>n=3</td>
<td>N/A</td>
</tr>
<tr>
<td>Saline</td>
<td>n=4</td>
<td>2 =20 minutes, 2=60 minutes</td>
</tr>
<tr>
<td>1 ug/kg CRF</td>
<td>n=5</td>
<td>60 minutes</td>
</tr>
<tr>
<td>2 mg/kg yohimbine</td>
<td>n=5</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Restraint</td>
<td>n=5</td>
<td>60 minutes</td>
</tr>
</tbody>
</table>

Once frozen, brains were sectioned on a cryostat to take a 500 um slice of the area where the nucleus accumbens is located. The NAcc was defined from 0.48-3.2 mm anterior to bregma, 6-7 D/V, and 1-2 M/L (Paxinos & Watson, 2006). Tissue was then manually dissected from the nucleus area from both hemispheres and placed into a microcentrifuge tube. The tissue was stored at -80 degrees C until the dopamine assay was completed.

In order to conduct the assay, the tissue was first combined with a diluted protease inhibitor (Enzo, BML-KI103-0001; 7X stock solutions in 1.5ml water) and PBS. The tissue was homogenized with an ultrasonic cell disrupter and then centrifuged 5 minutes at 5000 x g and the supernatant used for the protein and DA assays. Prior to the dopamine assay being conducted, protein was measured with a Bradford Protein Assay (Bio-Rad Bradford Protein Assay, 5000201). Briefly, 50 µl of standards and 50 µl of samples were pipetted into the plate with duplicates and 50 µl of the protein assay dye reagent. All samples were read on a BioTek
Cytation 5 microplate spectrophotometer. If samples fell above the standard curve, the sample was diluted and assayed again.

Dopamine was measured with a commercial kit from Enzo (Dopamine ELISA Kit, ENZ-KIT188-0001; detection range: 1.56-100 ng/mL; sensitivity: < 0.938 ng/mL), according to the manufacturer’s directions. Briefly, the samples were centrifuged and 50 ul of each standard or sample was added to the appropriate wells. Immediately after this, 50 ul of the antibody working solution was also added to each well. Then the plate was covered and incubated for 45 minutes at 37 degrees C. After this, the solutions were discarded and the plate was washed three times. A working solution was then added to each well and the plate was left to incubate for 30 minutes at 37 degrees C. After the time had elapsed, the solution was discarded and the plate was washed five times. Finally, a substrate was added to each well and it once again was allowed to incubate in the dark for 15 to 20 minutes at 37 degrees C. The final step was adding the stop solution to each well and then the plate was read within 20 minutes at 450 nm wavelength filter.

**Free Feeding Test**

To confirm that the pharmacological manipulations in Experiments 1 and 2 are not changing appetite or food preference, a free feeding test was conducted for restraint stress and yohimbine, as these two treatments were the only treatments to decrease performance. The data from the free feeding task helped to interpret the results in the operant FR5/choice task. In the free feeding task, a rat was injected with the same drug dose for the behavioral test or exposed to one hour of restraint stress. Following the appropriate lead time for each manipulation, the rat was then placed in a standard housing cage without bedding (17 inches x 13 inches) that had lab chow and sucrose pellets—the same two foods that they had access to in the operant procedures.
The rat had 30 minutes to consume as much of each food as they wanted with no work required to access either food. Each food was weighed before and after the session to measure the amount that was eaten.

**Statistical Analyses**

For the behavioral experiments, total number of lever presses and chow consumption was recorded and analyzed in SPSS (Version 25, IBM). As animals were weighed daily, the average body weight across drug testing days was used as a covariate for all animals and the stage of estrous cycle for female rats only on drug testing days was analyzed as a covariate for all statistical analyses. Body weight was a significant covariate for some of the analyses below and this is indicated in the discussion of those specific analyses; however, estrous cycle was not a significant covariate for any of the data, and therefore was not included in any of the analyses below. Training data for both Experiment 1 and 2 was analyzed with repeated-measures ANOVA across all days of training to assess stability of responding. Each day was compared to the proceeding day to see when stability in responding began. For behavioral data in Experiment 1, a 2 (sex) x 5 (stressor plus vehicle/control conditions) repeated-measures ANOVA was conducted. For behavioral data in Experiment 2, a 2 (sex) x 3 (vehicle + restraint, antalarmin + restraint, CRF) repeated-measures ANOVA was conducted. For free feeding analyses, a one-way ANOVA (pellets/chow by conduction) was conducted to compare pellet consumption across treatment groups as well as chow consumption across treatment groups. Follow-up comparisons were also conducted to compare each sex separately. For all analyses in both experiments, Greenhouse-Geisser correction to the degrees of freedom was used to correct for all Mauchley’s test of sphericity assumption violations. Planned post hoc comparisons (Bonferonni) were
conducted in order to compare each treatment with the vehicle and baseline conditions. The last three days of training were averaged and used as a control to compare to all stressors.

Finally, in Experiment 3 dopamine was first analyzed by averaging the duplicate samples. Then, further calculations were done by dividing the dopamine content by the protein weight for each sample. Samples that had non-detectable dopamine levels (i.e. below the limit of detection for the assay) were not included in the subsequent analyses. The animals that had no detectable dopamine levels were baseline animals (n=6); thus, they were not included. Dopamine data was analyzed by a one-way ANOVA test. If a significant f-statistic was found, post-hoc analyses were conducted to further understand differences between treatments. Outliers were removed from all data if the data was more than two standard deviations away from the mean.
CHAPTER FOUR: RESULTS

Experiment 1

The purpose of Experiment 1 was to examine the impact of acute stressors on effort-related decision making. Animals were first trained on autoshaping procedures and then were moved into FR5/chow choice training. Animals were initially required to learn the FR5 portion (5 days) and then chow was introduced into the chamber for 2 weeks or until stability was reached. Then, animals were exposed to acute stressors followed by free feeding procedures. Estrous cycle was tracked throughout stress testing.

Training Before Chow Introduction

Prior to the introduction of chow into the operant chamber, animals were trained on the FR5 program alone for five days to learn this aspect of the task. This program requires animals to press the lever five times for a one-pellet reward with unlimited rewards within a 30-minute time limit. For FR5 training, total number of lever presses prior to chow introduction were analyzed to investigate whether sex affected training and to assess the stability in responding overall. Body weight was not a significant covariate for training data at any stage of Experiment 1; thus, these analyses are reported without it. There were significant main effects of training day and sex on total number of lever presses \([F(4, 184)=45.2, p<0.001; F(1, 46)=13.28, p<0.001\), respectively]. Further, there was a significant training day by sex interaction, with males increasing the total number of lever presses to a greater extent compared to females \([F(4, 184)=3.061, p=0.024\]. Number of lever presses was stable by the last three days of training, as indicated by no significant difference across days three, four, and five \((p>0.05, \text{n.s.}; \text{Figure 4})\).
Figure 4: The first five days of FR5 training prior to chow introduction. Analysis was conducted to determine if stability in training was present prior to moving onto the next stage. There was a significant effect of training day and sex; however, all animals were stable by the end of the 5-day training period (n=48; 24 males, 24 females; *=p<0.05 from the preceding day).

Training After Chow Introduction

The number of lever presses and chow consumption were analyzed during the training period after the choice food introduction (total of 12 days) to investigate any differences in performance between sexes and the stability in responding. There were significant main effects of training day and sex on total number of lever presses [training day: F(11, 506)=14.593, p<0.001; sex: F(1, 46)=8.191, p=0.006]. While there were significant differences between training days early in the training period, there were no significant differences between days 15 through 17 (p>0.05, n.s.), indicating that performance was stable at this point in training. There was a significant interaction between training day and sex similar to training before chow, demonstrating a different pattern of chow consumption over the training period for males and females [F(11, 506)=2.827, p=0.005; Figure 5A). Post hoc analyses comparing sex on each day
of training indicated that, while there were significant differences between the two sexes across the earlier training days (days 6-13, 15), by the last two days of training, no sex differences were observed (p>0.05, n.s.).

There were significant main effects of training day on chow consumption [F(11, 506)=47.490]. As was the case with total number of lever presses, there were no significant differences from day 15 through day 17 of training (p>0.05, n.s.; Figure 5B) for chow consumption, indicating that chow consumption was stable by the end of this training period. Unlike the total number of lever pressing data above, there was no significant interaction [F(11, 506)=0.768, p=0.576] or main effect of sex [F(1, 46)=0.996, p=0.323].

**Stress Testing**

As body weight and stage of estrous cycle were not significant covariates on testing days, the following analyses were conducted without the covariates included. The total number of lever presses was significantly altered by acute stress exposure in the FR5/chow choice task [F(3, 138)=62.448, p<0.001]. There also was a significant main effect of sex [F(1, 46)=4.896, p=0.032] and a significant interaction between the stressor condition and sex, indicating that behavioral outcomes after stress exposure are sex-dependent [F(3, 138)=6.668, p=0.002; Figure 6A].

Post hoc analyses were conducted to compare the total amount of lever pressing under baseline (average of three days of training prior to any stress exposure), vehicle injection, restraint stress, and yohimbine injections conditions. They were also conducted to compare differences in sex within each condition. Overall, there was no significant difference between baseline and vehicle injection on lever presses (p>0.05, n.s.). However, the number of lever
Figure 5: Training days after chow introduction when the rat has access to lever pressing for banana flavored pellets and chow. Number of lever presses (Figure 5A) and chow consumption (Figure 5B) are shown. Analysis was conducted to determine if stability in training was present prior to moving onto the next stage. There was a significant effect of training day and sex on lever pressing and a significant effect of training day on chow consumption; however, all animals were stable by the end of the training period (n=48; 24 males, 24 females; *=p<0.05 from the preceding day).
presses were significantly reduced after restraint stress or yohimbine exposure compared to baseline and vehicle conditions \((p<0.001)\). There was no significant difference between total number of lever presses after restraint stress compared to yohimbine, indicating that this aspect of performance is similarly decreased after exposure to either stressor \((p>0.05, \text{n.s.}; \text{Figure 6A})\).

Male and female rats significantly differed under restraint stress and water injections \((p<0.05)\) with females having fewer presses than males, but not following yohimbine or during baseline conditions.

Chow consumption was also significantly altered by acute stress exposure in the FR5/chow choice task \([F(3, 138)=8.899, p<0.001]\). However, there was no significant main effect of or interaction with sex on chow consumption \((p>0.05, \text{n.s.})\), indicating that this measure is not influenced by sex. There were no significant differences between baseline and vehicle injection chow consumption based on post hoc analyses \((p>0.05, \text{n.s.})\). Baseline and vehicle chow consumption differed from restraint stress \((p=0.031; p=0.012, \text{respectively})\), but not from yohimbine injection \((p>0.05, \text{n.s.})\), indicating that restraint stress may impact eating behavior overall \((\text{Figure 6B})\). Although there was not a significant effect of sex, an exploratory analysis was conducted where each sex was analyzed by itself. It showed that females were driving the significant reduction in chow intake after restraint stress and no significant change under any other condition; while males showed no significant differences in chow consumption across testing.

**Total Food Consumed**

The total amount of food consumed in the stress portion of Experiment 1 was examined to examine the results in greater detail. A \(2 \times 4\) (sex x stressor) repeated measures ANOVA was
Figure 6: Number of lever presses (Figure 6A) and chow consumption (Figure 6B) following acute stress exposure. There was a significant effect of stressor and sex on lever pressing and a significant effect of stressor on chow consumption (n=48; 24 males, 24 females; *=p<0.05 compared to baseline and water).
conducted. Values were determined by adding together the weight in grams of pellets consumed and the total grams of chow consumed in each condition. It was found that there was a significant effect of stressor on total food consumed \([F(3, 138)=60.897, p<0.001]\) with restraint and yohimbine reducing total food consumed in the test and a significant main effect of sex \([F(1, 46)=6.735, p=0.013]\) with males consuming more total grams of food than females. There was also a condition by sex interaction on total food consumed \([F(3, 138)=4.415, p=0.012]\), indicating that males and females responded to the acute stressors differently. This also was not dependent on weight, as weight was not a significant covariate in these analyses \(p>0.05\), n.s.

Overall, post hoc analyses indicated that baseline and water conditions differed from both stress conditions \(p<0.05\). However, in an exploratory analysis where males and females were analyzed separately, it was found that, while this pattern holds true for males, food consumption for females was significantly different in every condition when compared to baseline \(p<0.05\); Figure 7 and Table 2).

Table 2: Table demonstrating that choice (preference) did not change after acute stress. Pellets in grams and chow in grams that were consumed in the operant box were summed to find the total amount of food consumed, which could then be used to find the percentage of that total that pellet consumption made up.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th>Males</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chow</td>
<td>Pellets</td>
<td>Total</td>
<td>Percentage Pellets</td>
<td>Chow</td>
<td>Pellets</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.15</td>
<td>5.25</td>
<td>5.4</td>
<td>97%</td>
<td>0.13</td>
<td>5.75</td>
</tr>
<tr>
<td>Water Injection</td>
<td>0.08</td>
<td>4.32</td>
<td>4.4</td>
<td>98%</td>
<td>0.09</td>
<td>5.9</td>
</tr>
<tr>
<td>Restraint</td>
<td>0.21</td>
<td>2.92</td>
<td>3.13</td>
<td>93%</td>
<td>0.38</td>
<td>2.36</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>0.04</td>
<td>1.51</td>
<td>1.55</td>
<td>97%</td>
<td>0.12</td>
<td>2.78</td>
</tr>
<tr>
<td>Average (g)</td>
<td>0.12</td>
<td>3.5</td>
<td>3.62</td>
<td></td>
<td>0.18</td>
<td>4.1975</td>
</tr>
</tbody>
</table>
Figure 7: Proportion in grams of pellets and lab chow consumed during Experiment 1 in each condition, demonstrating the change of consumption after each stressor. Analysis was conducted to determine if stressors altered food consumption. Baseline data was the average of the three days of training prior to the start of stress testing. There was a significant reduction in total food consumed in restraint stress and yohimbine conditions compared to baseline and water, indicating that the stressors may be influencing eating behavior (n=48; 24 males, 24 females).
Experiment 2

Experiment 2 assessed the impact of CRF manipulation on effort-related choice behavior so that the circuitry underlying the behavior could be better understood. In Experiment 2, animals were trained on autoshaping procedures and then were moved into FR5/chow choice training. Animals were initially required to learn the FR5 portion (5 days) and then chow was introduced into the chamber for 2 weeks or until stability was reached. Then, animals were exposed to CRF manipulations followed by free feeding procedures. Estrous cycle was tracked throughout testing.

Training Before Chow Introduction

Body weight was not a significant covariate for training data at any stage of Experiment 2; thus, these analyses are reported without it. As was the case for Experiment 1, there were significant main effects of training day and sex on total number of lever presses \[F(4, 132)=58.859, p<0.001; F(1, 33)=11.660, p=0.002, \text{ respectively}\]. When the sexes were examined individually, it was found that both males and females had a significant difference between days 1 and 2 \(p<0.05\) but none of the other training days were significantly different from each other \(p>0.05, \text{n.s.}\). Number of lever presses was stable by the fourth day of training, as indicated by no significant difference across days four and five \(p>0.05, \text{n.s.}; \text{Figure 8}\).

Training After Chow Introduction

Number of lever presses and chow consumption were analyzed during the training period after the choice food introduction (total of 28 days) to investigate any differences in performance between sexes and the stability in responding. There was a significant main effect of training day on total number of lever presses \[F(27, 891)=4.112, p<0.001\] but the differences were stable by
day 27, when pairwise comparisons indicated no significant differences between 23 of the 28 days (p>0.05, n.s., Figure 9A). There was no significant main effect of sex in the lever pressing data during this stage of training [F(1, 33)=3.47, p=0.071]. There was also no significant training day by sex interaction [F(27, 891)=0.802, p=0.641].

Figure 8: The first five days of FR5 training prior to chow introduction. Analysis was conducted to determine if stability in training was present prior to moving onto the next stage. There was a significant effect of training day and sex; however, all animals were stable by the end of the 5-day training period (n=36; 18 males, 17 females; *=p<0.05 compared to the preceding day).

For chow consumption during training, weight was a significant covariate [F(1, 32)=4.27, p=0.047]; thus, the following analyses were conducted with the covariate included. There were no significant main effects of training day on chow consumption [F(27, 864)=0.861, p=0.518]; however, there was a main effect of sex on chow consumption [F(1, 32)=5.344, p=0.027]. There was no significant training day by sex interaction [F(27, 864)=0.586, p=0.729]. As was the case with total number of lever presses, there were no significant differences between 23 of the 28
Figure 9: Training days after chow introduction when the rat has access to lever pressing for banana flavored pellets and chow. Number of lever presses (Figure 9A) and chow consumption (Figure 9B) are shown. Analysis was conducted to determine if stability in training was present prior to moving onto the next stage. There was a significant effect of training day on lever pressing and with no significant effects on chow consumption; however, all animals were stable by the end of the training period (n=35; 18 males, 17 females; *p<0.05 compared to the preceding day).
days of training (p>0.05, n.s.; Figure 9B) for chow consumption, indicating that chow consumption was stable by the end of this training period.

Testing

After achieving stable responding, all rats received each condition in a counterbalanced order and with at least 5 days between each condition. The average weight across testing days was a significant covariate in the total number of lever presses [F(1, 32)=5.999, p=0.02]; thus, all analyses were conducted with the covariate included. In the full ANOVA, there was no significant effect of manipulation on total number of lever presses [F(4, 128)=1.77, p=0.139]; however, there was a significant main effect of sex [F(1, 32)=11.365, p=0.002]. There was no significant interaction between condition and sex [F(4, 128)=1.087, p=0.366; Figure 10A].

Weight was not a significant covariate for the chow consumption analyses [F(1, 11)=0.15, p=0.706] and was removed for the following statistical tests. There was no significant effect of condition on chow consumption [F(4, 132)=3.080, p=0.064] or effect of sex [F(1, 33)=1.762, p=0.193]. There was no significant condition by sex interaction [F(4, 132)=1.276, p=0.283; Figure 10B].

Free Feeding Control Task

A free feeding test was conducted on the stress conditions that caused a significant decrease in performance as measured by total number of lever presses to determine whether a specific condition altered food consumption, as distinct from effort. Yohimbine and restraint stress were the only two conditions that affected performance in the operant box and thus, these two conditions were tested in the free feeding task. Weight was not a significant covariate in
Figure 10: Number of lever presses (Figure 10A) and chow consumption (Figure 10B) following testing. There was no significant effect of condition or sex on lever pressing or chow consumption (n=35; 18 males, 17 females).
these analyses (p>0.05); thus, the following analyses were conducted without it included. A one-way ANOVA was conducted to compare baseline to restraint and yohimbine exposure in the task. It was found that there was a statistically significant difference between treatment groups in pellet consumption \[F(2, 57)=51.197, \ p=0.001\]. Post hoc analyses revealed that baseline differed from yohimbine (p=0.001) and restraint (p=0.001) pellet consumption. It was also found that yohimbine and restraint pellet consumption significantly differed from each other (p=0.001). When comparing chow consumption in the treatment groups, it was found that there was not a significant difference between groups \[F(2, 57)=1.302, \ p=0.280\].

When comparing pellet consumption across groups in males alone, it was found that there was a significant difference \[F(2, 29)=39.103, \ p=0.001\]. Post hoc analyses indicated that restraint (p=0.001) and yohimbine (p=0.001) significantly differed from baseline pellet consumption and also differed from each other (p=0.001). In males, there was no significant difference found in chow consumption \[F(2, 29)=0.494, \ p=0.615\]. When comparing pellet consumption across groups in females alone, there was a significant difference in pellet consumption \[F(2, 27)=22.134, \ p=0.001\]. Post hoc analyses revealed that restraint (p=0.004) and yohimbine (p=0.001) are significantly different from baseline and from each other (p=0.005). There was a significant difference in chow consumption in females as well \[F(2, 27)=5.607, \ p=0.01\] with post hoc analyses revealing a difference between baseline chow consumption and restraint chow consumption (p=0.008; Table 3; Figure 11).
Table 3: Free feeding data demonstrating the weight of pellets and chow consumed by both males and females in each condition as well as the percentage of this weight that was made up of pellet consumption.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Percentage</th>
<th>Males</th>
<th></th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chow</td>
<td>Pellets</td>
<td>Total</td>
<td>Pellets</td>
<td>Total</td>
<td>Pellets</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.14</td>
<td>5.25</td>
<td>5.39</td>
<td>97%</td>
<td>0.14</td>
<td>6.9</td>
</tr>
<tr>
<td>Restraint</td>
<td>0.02*</td>
<td>2.15*</td>
<td>2.17</td>
<td>99%</td>
<td>0.12</td>
<td>1.8*</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>0.11</td>
<td>3.83*</td>
<td>3.94</td>
<td>97%</td>
<td>0.22</td>
<td>3.93*</td>
</tr>
<tr>
<td>Total (g)</td>
<td>0.09</td>
<td>3.74</td>
<td>3.83</td>
<td></td>
<td>0.16</td>
<td>4.21</td>
</tr>
</tbody>
</table>

Estrous Cycle Tracking

While stage of estrous cycle was not a significant covariate in any of the analyses, it is important to note the number of females in each stage of the cycle during each condition (Table 4). While the majority of the females on test day were in diestrous, there were females in each stage of their cycle. Female rodents spend the majority of their cycle in diestrous (Lovick & Zangrossi, 2021) and thus the distribution is not unexpected but difficult to draw conclusions.

Table 4: The number of females in each stage of the estrous cycle during Experiments 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Raw Number</th>
<th>Percentage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diestrous</td>
<td>Proestrous</td>
<td>Estrous</td>
</tr>
<tr>
<td>Water</td>
<td>14</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Restraint</td>
<td>17</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>11</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Raw Number</th>
<th>Percentage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diestrous</td>
<td>Proestrous</td>
<td>Estrous</td>
</tr>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>
Figure 11: Free feeding test showing weighed amounts (grams) of banana flavored pellets relative to chow. Test was conducted to determine if stressors altered food consumption when the effort to have access to both food items was the same. Baseline data was collected the day prior to stress testing. There was a significant reduction in banana pellets consumed after restraint stress and yohimbine exposure with no change in chow compared to baseline overall (n=23 (yohimbine and baseline; 12 males, 11 females); n=12 (restraint; 6 males, 6 females)).
Experiment 3

Dopamine content in the NAcc of animals treated with CRF, yohimbine or restraint stress was compared to dopamine content in animals with no manipulation (baseline) and animals that were injected with saline. All animals used had not been through any behavioral training in order to assess the impact of each of the various stressors. After exposure, all animals were rapidly decapitated and brains were dissected. A two-way (sex x condition) ANOVA was initially conducted in order to compare dopamine in each group.

There was no effect of sex [F(1, 12)=0.135, p=0.72] and thus, males and females were collapsed and a univariate ANOVA was conducted. Overall, it was found that there was no significant main effect of condition on dopamine [F(4, 17)=1.023, p=0.424; Table 5]. It was also found that, while 28 duplicate samples were run in the dopamine assay, six produced zero values after calculations were conducted, which account for 21.4% of the total samples. The six samples were baseline samples (67% of the baseline samples) had non-detectable levels of dopamine.

Table 5: Dopamine levels (ug/ml) in the nucleus accumbens of rodents (males and females combined) in each condition tested. Mean and SE; no significant differences were detected (n=22; dopamine values = sample/dilution).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Saline</th>
<th>CRF</th>
<th>Yohimbine</th>
<th>Restraint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean + SE</td>
<td>4.66 ± 3.77</td>
<td>32.26 ± 11.28</td>
<td>34 ± 25</td>
<td>50.5 ± 11.98</td>
<td>36.3 ± 17.57</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
CHAPTER FIVE: DISCUSSION

The current study investigated the 1) influence of acute stress on motivated behavior as assessed through the FR5/chow choice task, 2) impact of manipulations of the CRF system on FR5/chow choice task behavior, and 3) effect of acute physical and pharmacological stressors on dopamine content in the nucleus accumbens. In Experiment 1, training data with and without chow in the operant chamber showed a significant increase in lever pressing across days. Interestingly, while there were sex differences present, by the end of training, the sexes were performing the task to a similar level. Acute stress exposure significantly impacted performance as measured by total number of lever presses for the banana pellet. Specifically, while there were no differences in lever pressing between baseline and following a vehicle injection, restraint stress and yohimbine decreased the total number of lever presses. Importantly, there was a significant interaction between stressor and sex, indicating that performance measured by total number of lever presses after stress exposure is sex-dependent. Females had fewer lever presses after stress compared to males, particularly after restraint stress. Within the stress conditions, chow consumption was reduced following restraint stress only in Experiment 1, indicating that restraint stress may be impacting feeding behavior generally (Figures 6A and 6B). This conclusion was further supported in the free feeding test with either an injection of yohimbine or restraint stress reducing sucrose pellet consumption but only females showed a reduction of chow consumption following restraint (Figure 11).
In Experiment 2, the same training was used with the FR5 schedule as in Experiment 1. Similar to Experiment 1, training data both before and after chow was introduced showed a significant increase in lever pressing across days. However, different than Experiment 1, males and females only differed by sex prior to the introduction in chow while this sex difference disappeared once chow was introduced into the chamber. Also different than Experiment 1, body weight was a significant covariate for chow consumption in Experiment 2. For both number of lever presses and chow consumption during this training period, pairwise comparisons showed that there were no significant differences across most days, indicating that performance was stable prior to CRF manipulations taking place (Figure 9A and 9B). Manipulations of CRF receptor had no effect on lever pressing or chow consumption. There were no differences in performance between baseline, vehicle, and CRF injections. While vehicle + restraint stress reduced the number of lever presses replicating data from Experiment 1, pretreatment with the CRF antagonist antalarmin did not attenuate those effects in the way that was predicted.

In Experiment 3, dopamine was measured in the NAcc after rats were exposed to saline, CRF, restraint or baseline. It was found that there was no significant main effect of condition on dopamine in the region. Further analyses revealed that there were no significant main effects of sex observed, indicating that this may not be a sex-specific change in the NAcc following acute stress. Interestingly, the dopamine assay was unable to detect dopamine levels in the baseline condition, suggesting that under basal conditions dopamine levels are very low in the nucleus accumbens of male and female rats. All other conditions had detectable levels of dopamine, which may indicate that dopamine was increased during these conditions including saline injection.
Influence of Acute Stressors on FR5/Chow Choice Behavior

Experiment 1 tested the effect of various acute stressors on the total number of lever presses and chow consumed during the FR5/chow choice behavior task. It was hypothesized that acute stressor would decrease responding for the more highly valued reward, with females showing a greater deficit compared to males. The study was designed to test whether acute stress affects discrete elements of motivated behavior – 1) choice of reward and 2) effort to procure the reward. As was expected, it was found that stress exposure significantly impacted performance as measured by total number of lever presses, specifically between the baseline/vehicle conditions (e.g. control) compared to restraint stress and yohimbine exposure. Based on this finding, it is clear that acute stress altered effortful behavior through its negative impact on the higher effort reward after yohimbine injections or restraint stress. Interestingly, while effort was clearly reduced after acute stressors, choice, or preference, was not changed to the same extent. The percent of pellets that were consumed in each condition (Figure 7) is stable, despite the large reduction in total pellets earned following restraint. Thus, generally, the major hypothesis for Experiment 1 was supported that acute stress exposure decreased the responding for the more effortful reward.

Activational aspects of motivation require effort or work in order for an organism to gain access to significant stimuli within its environment (Cofer & Appley, 1964). Because an animal is required to overcome obstacles to receive the desirable stimuli, they must allocate resources towards a stimulus or the effort required to obtain the reward (Salamone & Correa, 2012). Effort can be assessed through measures of the rate, persistence, and levels of behavior. This effort exertion may be brief (e.g., an organism quickly darting for food), but there are other times when effort must be sustained to gain access to the reward (e.g., an organism having to travel long
distances in order to obtain food; Salamone & Correa, 2012). Similar to the current findings, acute stress has been shown to disrupt effort. Male rats restrained for 20 minutes prior to the testing showed reduced lever pressing to obtain a sucrose pellet food reward under a progressive ratio schedule of reinforcement as measured by breakpoint (Wanat et al., 2013). Likewise, male rats exposed to restraint stress for one hour decreased preference for a more costly sucrose reward (Shafiei et al., 2012) and preference for a higher density reward (Bryce & Floresco, 2016). Taken together, these findings support the findings from the current study and suggest that acute stress can reduce the effort or activational component of motivated behavior.

Another aspect of motivated behaviors is choice or the directional aspects to behavior (Cofer & Appley 1964; Salamone, 1988). Organisms continuously make choices between multiple options, such as gaining access to food that is near a predator. Experimentally, this can be manipulated in several ways by offering animals a choice without any change in effort in accessing each reward. For instance, in the free feeding test, animals are offered the choice between consuming the preferred pellets or a standard lab chow with no real effort required to obtain either source of food (Errante et al., 2021; Nunes et al., 2013). This test is often used to assess changes in food preference and hunger, and has been used as a control for more complicated choice procedures (Errante et al., 2021; Nunes et al., 2013). In the current studies, acute stress did not appear to impact choice of food, such that in both the operant box and the free feeding test, the consumption of sucrose reward was still greater than chow. This is consistent with previous literature, which has shown that after exposure to acute stress, sucrose preference remains consistent as measured by a two-bottle sucrose test (Garcia-Keller et al., 2021).
In the current study, effort and choice/preference were examined by looking at the data from different perspectives. Very few researchers in this field consider both components of motivation when analyzing data: papers report whether effort is disrupted (e.g. lever pressing, latency to location) or food item preference is changed (e.g. sucrose preference test). We recently published a paper to examine food motivation analyzed in this way and found similar results to the present study in that, while total food decreased as a function of effort after a manipulation, preference did not change as measured by choice (Errante et al., 2021). Table 4 shows that while food consumption was lowered after stress in the operant box due to lowered lever pressing, the food that was eaten and worked for was preferentially still the banana pellets. Clinically speaking, approaching motivation in this way is essential, as it informs treatment methods for disorders characterized by a disruption in effort expenditure.

Sex Differences in Motivated Behavior

Throughout the procedures, there were differences in lever pressing behavior between male and female rats. This finding supports the developing literature characterizing sex differences within effort-related decision making procedures. Previous studies utilizing effort-related procedures have shown that sex differences exist within these types of motivational tasks (Errante et al., 2021; Presby et al, 2019; Presby et al., 2020). Once chow was introduced into the operant chamber, performance continued to change across days for both males and females, as indicated by a significant interaction between training day and sex with males showing a greater number of total lever presses compared to females. Errante and colleagues showed sex differences in the training of male and female rodents prior to and after the introduction of choice food in a progressive ratio/chow choice task, which required ratio increases progressively (Errante et al., 2021). While this sex difference was seen across several of the variables that were
measured in the task, it was notably seen within the total number of lever presses across all training data (Errante et al., 2021), which is similar to the findings from the current study demonstrating a sex difference in effort-related behavior (Figures 4 and 5). In the present study, there was a significant sex difference and a significant sex by training day interaction, demonstrating that, while both males and females learn the task at hand as indicated by stability in responding the last two days of training prior to chow introduction, males’ bar pressing increased faster over training days. Taken together, the results of the training data help to support a growing body of work that points to sex differences in not only motivated tasks, but effort-based tasks as well.

Previous research has also shown sex differences in behavior within effort-related decision making tasks. In a different approach to assessing effort-related decision making, one study used a T-maze to allow an animal to choose between voluntary physical activity (wheel running) or to consume standard lab chow to better understand the psychomotor symptoms and fatigue associated with depression (Presby et al., 2019). During testing, male and female rodents were placed in the apparatus for 30 minutes per day and were able to move between the two options. It was found that female rodents spent more time running on the wheel and less time consuming standard lab chow while male rodents showed the opposite behavioral profile (Presby et al., 2019). Collectively, these results may suggest that perhaps males are more motivated by food regardless of the effort associated with accessing that food source, whereas females may be interested in other activities such as running wheel. In the current study, even though sex differences are minimized by the end of chow training, males still lever press more than females, which goes against the original assertion that the FR5 task would not produce sex differences. Although there may be different activity preferences initially between male and female rodents
(Presby et al., 2019), the current findings suggest that females’ effort for food reward eventually reaches similar levels to males, as indicated by the minimization of sex differences as the experiment goes on. Many studies have demonstrated that females will work for food when food is the only option they can receive (Presby et al., 2020; Errante et al., 2021; Sanders et al., 2019; Yoest et al., 2019); however, it is important to consider the possibility that the preferred activity of female rodents may not be associated with food when interpreting studies exploring sex differences.

Although previous research has shown that food restriction increases responding for a sucrose pellet in operant procedures (Errante et al., 2021; Yohn et al., 2016; Randall et al., 2012; Nunes et al., 2013), chronic food restriction was not used in the current study because preliminary data indicated that it was not necessary to motivate behavior and it has been used as a chronic stressor in prior research (Marinelli et al., 1996; Sedki et al., 2013). Further, while much work has been done in male rodents with food restriction to increase performance in an operant task (Randall et al., 2012; Yohn et al., 2015, Yohn et al., 2016; Errante et al., 2021), food restriction in female rodents does not consistently increase their performance in all types of motivated tasks. For instance, food restriction in female hamsters reduced food-seeking behavior in a conditioned place preference test, rather than enhancing it (Klingerman et al., 2011). Food restricted female California mice in the Barnes maze showed increased latencies to locate the target home during acquisition, but decreased latencies during reversal testing (Steinman et al., 2011). Preliminary data from our laboratory using a different progressive ratio procedure indicated that female rats fed ad libitum outperform males under the same food conditions (Anderson, 2017); however, when both males and females are food restricted, the performance of male rats increased with no change in the performance of females (unpublished data).
Collectively, these findings indicate that future research should consider whether food restriction is useful or necessary as a method to increase performance or motivation in assessing motivated behavior of female rodents. While food restriction may increase baseline responding, thereby showing a larger deficit after a manipulation, the stressors in the current study already significantly reduced effort and the direction of this change would more than likely not be impacted by food restriction; however, sex differences may change with food restriction, which should be considered in future studies.

In addition to it being unnecessary to restrict food availability to elicit the effort required by the FR5 choice task, food restriction has served as a chronic stressor in published research (Lenglos et al., 2013; Marinelli et al., 1996; Sedki et al., 2013; Krieger, 1974). For female rats, food restriction reduced the consumption of palatable foods versus standard foods following exposure to chronic restraint stress in combination with repeated food restriction (Lenglos et al., 2013). Likewise, our research found that female rats under food restriction conditions demonstrated reduced motivation for a palatable reward when effort is required, while the females continued to show preference for sucrose pellets over standard laboratory chow in the free feeding task (Errante et al., 2021). In addition, food restriction increases stress hormones, particularly in female rodents (Krieger, 1974). One study found that with intermittent food restriction where food was restricted two days per week, female rats had elevated plasma corticosterone levels compared to both females that were not food restricted and food-restricted males (Lenglos et al., 2013). Interestingly, when our laboratory looked at food restriction as a stressor, binge-like response in animals were observed (unpublished data), which is consistent with some previous literature in humans that suggests that HPA axis hyperactivity can lead to binge eating (Gluck et al., 2004). Thus, while food restriction in the current study may have
increased lever pressing during training, the potential negative impact on female rodents combined with the preliminary data were convincing that it was not needed but it may be interesting to manipulate chronic food restriction to create a higher response rate before testing with acute stress.

There were sex differences in the amount of lab chow consumed once chow was introduced into the operant chamber during training. Specifically, there was a clear spike in chow consumption during the first day of testing, which quickly dropped and remained relatively low for the rest of the training period except for days five and six (Figure 5B). Although males ate more lab chow compared to females throughout the study, both sexes consumed more banana pellets in comparison to chow (Table 2), which supports the preference for the banana pellet food reward compared to the lab chow. Lever pressing performance remained elevated across all of the training days, indicating that the banana pellet reward is the preferred food source. The free feeding data from the present study also shows that banana pellets are consumed to a greater extent than they were in the operant box, even though the same stressor is being presented (baseline in operant box: 5.8 grams (males), 5.3 grams (females); baseline in free feeding: 6.9 grams (males), 5.3 grams (females); yohimbine in operant box: 2.4 grams (males), 2.9 grams (females); yohimbine in free feeding: 3.9 grams (males), 3.8 grams (females); restraint in operant box: 2.8 grams (males), 1.5 grams (females); restraint in free feeding: 1.8 grams (males), 2.2 grams (females); Figure 11). In the free feeding task, both males and females consumed significantly fewer pellets following restraint stress and yohimbine exposure compared to baseline; however, only females reduced their chow consumption when comparing baseline to restraint. Body weight was not a significant covariate in these analyses, indicating that these differences are not due to differences in weight and metabolic need under the stress conditions.
Females overall ate less after restraint stress, even when no effort was required to consume either food source in the free feeding task. This further indicates a sex-specific effect of restraint stress, not dependent upon the value of the food item. Taken together, these findings demonstrate that, while males consume more lab chow in comparison to females, the banana flavored pellets are still the preferred food source for both sexes.

Finally, the training data demonstrate greater differences at the beginning of training compared to the end, suggesting that females may take longer to reach the performance levels of males but can/will achieve the same level. This pattern is similar to other studies from the lab that used a different type of effort-related decision making task (Errante et al., 2021), as well as other behavioral measures (Safari et al., 2021; Dalla et al., 2008; Cimadevilla et al., 2004). The previous data using a progressive ratio schedule found a sex difference in training before and after chow introduction, similar to the current study. However, the past work in the lab found that when a manipulation was done, the sex difference disappeared, indicating that males and females were impacted in a similar way by the pharmacological manipulation used in the study (Errante et al., 2021); however, this is not the case in the current study. The current study found a sex difference during the acute stress conditions, with post hoc analyses indicating that females had a greater decrease in lever pressing following restraint stress. Considering sex differences were not present in baseline or vehicle conditions, the sex differences seen in the current study and those seen in previous studies are due to the addition of acute stressors. Although not all acute stressors in the current study demonstrated sex differences, future work should assess what about acute stressors produces a sex difference in comparison to other manipulations, particularly as it concerns effort-related decision making tasks.
Impact of Acute Stress on Motivated Behavior

Stress triggers multiple neural and endocrine systems to allow an organism to respond appropriately to the challenge to homeostasis and wellbeing (Hollon et al., 2015). In the present study, restraint stress led to a reduction in total number of lever presses compared to baseline and vehicle injections but no increase in chow consumption (Figure 6A and 6B). Previous studies that have utilized the FR5/chow choice procedure observed a decrease in the total number of lever presses, and then an increase of chow consumption, representing a compensatory reallocation of behavior through which the animal’s hunger needs are still being fulfilled. The rat no longer exerts the effort for the highly preferred pellets and will seek out an alternative food source (Salamone et al., 1991, 2002; Koch et al., 2000; Nowend et al., 2001; Sink et al., 2008; Farrar et al., 2010; Nunes et al., 2013). However, this is not the behavioral profile that was present after exposure to restraint stress in Experiments 1 or 2. While total number of lever pressing was decreased, the compensatory increase in chow consumption was not present (Figure 6A and 6B). These findings may indicate that restraint stress caused a reduction in eating regardless of the food source and/or effort required to obtain the food. This is supported by the free feeding test, which showed that, even when effort requirements between the banana flavored pellets and the lab chow were equal, there still was a reduction in both after the animals had experienced restraint stress.

This finding supports previous research demonstrating a reduction in reward seeking behavior following stress (Ironside et al., 2018; Willner, 2017). In a similar study, male rats restrained for 20 minutes showed reduced lever pressing to obtain a sucrose pellet food reward under a progressive ratio schedule of reinforcement as measured by breakpoint (Wanat et al., 2013). When focusing on a choice behavioral paradigm, there are very few studies that have
looked at restraint stress, but they have shown similar findings. Male rats tested in an operant-based procedure with a low effort/low reward option of two pellets while the high effort/high reward option produced a reward of 4 pellets found that restraint stress lowered the preference for the high effort/high reward option with longer choice latencies on the high reward lever (Bryce & Floresco, 2016; Shafiei et al., 2012). Taken together, these findings help to support the findings from the current study, which suggest that acute restraint stress reduces motivated behavior for palatable food rewards.

It is possible that the restraint-induced reduction in motivated behavior may be indicative of more general change in locomotion. The increase in choice latency after restraint stress may suggest the animals are simply moving slower throughout the task after being exposed to a stressful situation that is outside of their control (Shafiei et al., 2012). Although not always a consistent finding, other studies have reported a reduction in locomotor activity as measured by the open field test after restraint stress (Masood et al., 2003; Drouet et al., 2015). Specifically, in one study, it was found that after 1 hour of restraint stress, there was decreased ambulation and rearing in the open field test in male rodents (Masood et al., 2003). In the current study, a delay was given to animals in between the end of restraint stress and operant testing, which was done in an effort to reduce the impact of acute effects on locomotion and grooming on lever pressing behavior; however, it is possible that the delay may not have been long enough to fully reduce the acute effects of restraint stress on locomotion. Thus, it is conceivable that the reduced food consumption overall in both the free feeding and operant box conditions could be due to changes in motor activity, which should be tested in future studies.

In the current study, yohimbine significantly reduced the number of lever presses compared to baseline and water conditions (Figure 6A and 6B). Although not significant,
yohimbine administration also led to an increase in chow consumption, which is comparable
to other studies that have utilized a choice paradigm (Salamone et al., 1991, 2002; Koch et al.,
2000; Nowend et al., 2001; Sink et al., 2008; Farrar et al., 2010; Nunes et al., 2010). In one study
with similar procedures, administration of a dopamine D1 antagonist shifted behavior from lever
pressing to consuming significantly more chow (Nunes et al., 2010). Other work has been done
to determine which neurotransmitter system mediate yohimbine’s actions on behavior.
Yohimbine administered with different receptor antagonists, including a CRF antagonist, a
glucocorticoid antagonist, three noradrenergic antagonists, a dopamine antagonist, an opioid
antagonist, and a serotonin antagonist failed to attenuate yohimbine’s decrease of impulsive
action, as measured by premature responding in an operant box task, suggesting that yohimbine
interacts with multiple neurotransmitter systems (Mahoney et al., 2016). Overall, while
yohimbine’s behavioral effects resemble those of a D1 receptor antagonist, future studies should
further examine other neurochemical systems to further interpret the present findings.

The findings of the current study in regards to the ability of yohimbine to act as a
pharmacological stressor are quite unique compared to other studies. Yohimbine activates the
norepinephrine system, which activates the HPA axis (Plotsky et al., 1987). As was previously
mentioned, it is used to study impulsivity in animals due to its activational effects on the
catecholamine systems, both in drug naïve animals and in animals previously exposed to elicit
drugs. In one study focused on cue contingency during food self-administration, yohimbine
induced reinstatement of lever pressing after extinction training, an effect that is very common in
reinstatement studies (Le et al., 2005; Lee et al., 2004; Shepard et al., 2004; Chen et al., 2015).
Many studies have used yohimbine as a stressor to test reinstatement of drug seeking behavior
following extinction training. For example, rats trained to self-administer heroin (Banna et al.,
methylamphetamine (Shepard et al., 2004), and cocaine (Brown et al., 2012), were then challenged with yohimbine as a pharmacological stressor to assess stress-induced reinstatement of drug seeking. Importantly, yohimbine is an alpha-2 adrenergic antagonist, allowing it to directly inhibit presynaptic receptors and leading to a stress-like response in the body through stimulation of norepinephrine (Abercrombie et al., 1988; Aghajanian & VanderMaelen, 1982), which is different than other stressors such as restraint stress that directly act on stress hormones like CRF (Lombardo et al., 2001). The difference in the pharmacological actions between the two stressors may account for the behavioral differences seen in the choice task in the present study. Specifically, yohimbine’s actions on the norepinephrine system could lead to a lever pressing deficit based on a reduction of effort due to the relationship between dopamine and norepinephrine (Varazzani et al., 2015); however, restraint produces an overall deficit in eating behavior because of the direct actions on the CRF system. Taken together, these findings may suggest different pharmacological treatments for motivational dysfunction, depending upon the specificity of the behavioral deficits.

Influence of CRF Manipulations on FR5/Chow Choice Behavior

In Experiment 2, a separate group of rats underwent testing to determine the importance of the CRF system in choice behavioral deficits. Based on previous studies, it was hypothesized that CRF, a peptide associated with stress, would decrease effort for a palatable food reward as a measure of less effortful/hypomotivated behavior (Yohn et al., 2016; Randall et al., 2012; Bryce & Floresco, 2016). It was also predicted that this effect would be more pronounced in females based on previous data from our lab and others (Errante et al., 2021; Heck & Handa, 2019; Palumbo et al., 2020). Given the finding in Experiment 1 that demonstrated that restraint stress reduced lever pressing for the sucrose reward, Experiment 2 predicted that administration of the
CRF antagonist prior to restraint stress would attenuate the decrease in effort for the palatable food reward but not for choice food of standard rat chow. Previous studies have shown that administration of CRF antagonists reverses effort-related impairments seen after stress (Bryce & Floresco, 2016). Although it was predicted that the effect of restraint stress would be more pronounced in females, the dose of CRF chosen was based on the only available studies that were used male rats and it was expected that antalarmin would return lever pressing of both males and females to baseline levels. Overall, it was found that restraint stress in both conditions significantly impaired performance similar to Experiment 1, with no attenuation provided by the CRF antagonist antalarmin. Additionally, Experiment 2 demonstrated that males showed higher lever pressing in all conditions. While the data replicated Experiment 1 in that restraint stress reduced effort for, but not choice of, a food reward, injection of drugs targeting the CRF peptide system did not alter motivation as assessed by the FR5 task.

Restraint stress has long been known to increase circulating glucocorticoids with many studies characterizing the adrenal hormone corticosterone. These stress hormones are thought to be a potential target for the long-term effects of restraint stress in rodents and indicative of HPA axis responsiveness (Dal-Zotto et al., 2002). Several studies have pointed to CRF as a key player in the impact of restraint stress. Prior research has shown that restraint stress increases the release of CRF, which occurs immediately after the onset of restraint stress (Pich et al., 1995; Merali et al., 1998). The increase in the release of CRF activates the HPA axis, which then can lead to the increase in the pituitary and adrenal hormone corticosterone and ultimately creating a major stress response for the animal. While no study could be found that examines all of this together, it is of interest, particularly when considering the data from the current study that clearly demonstrates a behavioral change after restraint stress. It is possible that restraint stress increased
CRF in the current study and that the dose of antalarmin was not high enough to counteract these effects. Future studies should explore this in greater detail, as it would help to explain the role of CRF in restraint stress generally.

Unexpectedly, CRF did not alter performance in the task nor did the CRF antagonist reduce the effects of acute restraint stress. While prior research indicates that administration of CRF should mimic the effects of stress (Bryce & Floresco, 2016) and the CRF antagonist can block the restraint-induced changes in behavior (Wanat et al., 2013), it is possible that these CRF is not critical for effort-related decision making task. One previous study assessed CRF in an effort-related decision making task but unlike the paradigm used in the current study, the task required animals to lever press on a low reward lever and a high reward lever for the same food source, whereas the current study had two different food sources that had different actions required to obtain them (Bryce & Floresco, 2016). Given that our hypothesis was based on the Bryce and Floresco (2016) study, it is surprising that the current results differed. While additional past research also has indicated roles for CRF compounds in motivated behavior (Koob, 2010; Hupalo et al., 2019), effort-related choice procedures are unique; thus, it is possible that the procedural differences outlined below accounts for the current findings.

A likely difference between the studies is the route of drug administration, with the prior research infusing CRF and the CRF antagonist directly into the brain while the current study administered both intraperitoneally. Specifically, Bryce and Floresco (2016), infused CRF and a CRF antagonist intracerebroventricularly or CRF was also infused into the VTA prior to testing in an effort-related decision making task. The study suggested that CRF could regulate mesolimbic dopamine at the level of the VTA. The differences in route of administration are a likely reason for the current study not supporting the previous research. Additional studies have
utilized systemic dosing of CRF with positive results as indicated by significant increases in CORT and ACTH (Lovelock & Deak, 2020). The literature suggests that only small amounts of exogenous CRF cross the blood brain barrier (Kastin & Akerstrom, 2002), reducing the effective dose in the brain in the current study. Because of this, future studies should explore a similar procedure but with infusions of CRF directly in the brain to elucidate the connection between dopamine and CRF in effort-related tasks. Additionally, future studies should contemplate a dose response curve for both CRF and antalarmin, especially considering both male and female rats. While dosing for both drugs was based on previous studies (Loveleak & Deak, 2020; Furman, 2017; Zorrilla et al., 2002; Ducottet et al., 2003), higher doses may be needed to get the desired effect if administered systemically; thus, future studies should examine dosing as a potential factor in the interpretation of the current results related to CRF. However, the current data is still important in terms of treating depression as most pharmacological approaches to addressing motivational dysfunction depend upon a systemic administration.

NAcc Dopamine Levels After Stress Exposure

In Experiment 3, dopamine content in the NAcc was measured in males and females that were exposed to the stressors in Experiments 1 and 2. It was predicted that there would be increased measurable dopamine in the NAcc after exposure to the stressors compared to baseline animals and animals injected with saline. It was also predicted that dopamine would be elevated to a greater extent in females compared to males, as previous studies indicate that females are more sensitive to stress (Babb et al., 2013; Iwasaki-Sekino et al., 2009). However, while an overall elevation was expected, it was predicted that this might be stressor specific. Overall, there was no significant increase in NAcc dopamine levels following acute stress with no significant main effect of sex.
Although perhaps it should have been predicted, the low levels of dopamine in the NAcc were not detectable with the microplate assay. Without dopamine in control groups, it is difficult to expect significant changes in dopamine in Experiment 3. Most dopamine-containing neurons are clustered into three groups, one of which is the mesolimbic system with dopamine cell bodies in the VTA (German et al., 1983; Arsenault et al., 1988). The VTA projects to the NAcc and the content of dopamine as analyzed by the microplate assay would be limited to the nerve terminals and synapsis within the NAcc. Dopamine levels in the nucleus accumbens tissue is low, approximately 40.6 pg/20 ul compared to other brain regions, such as the striatum (37.8 pg/20 ul; Abercrombie et al., 1989). Other experimental approaches may be more sensitive to low dopamine levels, such as high performance liquid chromatography or Western blots.

Yohimbine did not significantly alter dopamine levels in the NAcc tissue compared to baseline in either male or female rats. This finding is inconsistent with our predictions and the little prior research that measured dopamine following an injection of yohimbine in rats (Chen et al., 2015; Cifani et al., 2010). The only study that has examined dopamine after yohimbine exposure used microdialysis to compare baseline dopamine in the nucleus accumbens and prefrontal cortex (Chen et al., 2015). Both the prefrontal cortex and the NAcc had higher levels of dopamine compared to baseline with the prefrontal cortex being more elevated after yohimbine administration as compared to the nucleus accumbens (Chen et al., 2015).

Microdialysis assessment of dopamine levels differs from dopamine content as measured through tissue dissections. Specifically, dopamine measured through microdialysis is estimating dopamine by sampling the synaptic and extrasynaptic regions of a brain region rather than measuring intracellular and extracellular components as in tissue dissections that were done for the current study. Many stressors including yohimbine and restraint increase dopamine as
measured by in vivo microdialysis (Chen et al., 2015; Abercrombie et al., 1988; Tidey et al., 1996; Gambarana et al., 1999) and this increase is considered part of the fast stress responsive system. Considering this, future studies may want to investigate yohimbine on dopamine in the nucleus accumbens through different approaches, such as through microdialysis or electrophysiology.

Similar to yohimbine, CRF was hypothesized to increase dopamine in the nucleus accumbens due to its role in the HPA axis. Based on a previous study that examined the interaction between dopamine and CRF in a progressive ratio operant task by infusing a CRF antagonist bilaterally into the VTA and then were put into 20 minutes of restraint stress (Wanat et al., 2013). While the stressor significantly reduced the breakpoint in the task compared to baseline, this was attenuated by administration of the CRF antagonist directly into the VTA in a dose-dependent manner (Wanat et al., 2013). Interestingly, the study also examined dopamine release after CRF administration in the VTA using fast-scan cyclic voltammetry during the progressive ratio task and found that motivation was reduced with CRF administration and dopamine release was inhibited in response to reward delivery (Wanat et al., 2013). Another study examined the role of CRF in the NAcc through fast-scan cyclic voltammetry (Lemos et al., 2012). CRF administered to naïve mice increased dopamine in the NAcc through activation of either CRF receptor subtypes; however, when animals were exposed to severe stress, this effect was abolished with no recovery seen for at least 90 days (Lemos et al., 2012). Taken together, these findings indicate a complex stress circuitry between CRF and dopamine; thus, future studies should consider specifically targeting dopamine-rich areas of the brain with microinjections of CRF or CRF antagonists to understand this circuit more specifically.
Several studies have examined the relationship between dopamine and restraint stress (Carlson et al., 1991; Imperato et al., 1992; Puglisi-Allegra et al., 1991). One study found that there was an increase in dopamine and its metabolites as measured through microdialysis during restraint for the first approximately 60 minutes; however, after this point, dopamine gradually decreased to basal levels (Puglisi-Allegra et al., 1991). While animals in Experiment 3 were exposed to 60 minutes of restraint stress, which is similar to the timeline of the previous study, it is possible that the peak dopamine response was missed and dopamine levels were declining. Further, blocking CRF with an antagonist did not attenuate restraint-stimulated increases in dopamine suggesting at least the final output of the HPA axis may not be important in stimulating nucleus accumbens dopamine (Puglisi-Allegra et al., 1991). Additional research may target the timing of restraint in comparison to measuring dopamine in the NAcc as dopamine levels were trending towards significance following restraint compared to control dopamine levels (Table 5). Further, future studies may wish to increase the power for NAcc dopamine analysis. The current study had more than half of the control animals with undetectable levels of dopamine; had there been more power, perhaps the results in comparison to baseline would have been different. While the resulting data points to a potential connection between dopamine and restraint stress, further studies need to examine this relationship in greater detail to further the understanding of the relationship between stress, CRF, and the mesolimbic system.

The design of the experiment allowed for the novel comparison of the mesolimbic system in female and male rats following acute stress or CRF. Based on the data, there were no sex differences in dopamine content in the NAcc, suggesting the relationship between stress and the mesolimbic system are not dependent upon sex. This was surprising following stress exposure as prior research has shown sex differences with dopamine and stress manipulations. In one study,
male and female rats received injections of a dopamine D2 antagonist, a D2 agonist, antalarmin (CRF antagonist used in current study), or yohimbine and then were tested in a rat version of the Iowa gambling task assessing decision making (Georgiou et al., 2018). Interestingly, it was found that each drug had a sex-specific effect. The D2 antagonist decreased advantageous responding in males but not females, while the D2 agonist did the opposite (Georgiou et al., 2018). Yohimbine also decreased advantageous responding in female rats in a dose-dependent manner while males were only impacted at higher doses and antalarmin only increased optimal choice in female rodents (Georgiou et al., 2018). The behavioral responses to the pharmacological manipulations suggested that there were sex differences in decision making, which were dopaminergic- and stress-dependent. Many prior studies that have demonstrated sex differences after stress exposure. In one study, male and female rats exposed to 30 minutes of restraint stress resulted in a HPA axis hormonal response in all animals, although it was much higher in females (Babb et al., 2013). In another study, females had a heightened response to a 60 minute footshock or psychological stress induced by a communication box in both male and female rodents as measured by CRF gene expression as well as ACTH and CORT secretion while males showed no such elevation (Iwasaki-Sekino et al., 2009). While previous studies have found sex differences in various measures similar to the current study, it is possible that the experimental parameters for the stress and stress hormone were not optimal for detecting sex differences in dopamine measures. Future studies could explore different targets for sex differences, such as the prefrontal cortex known to be quite sensitive to stress and rich in dopamine projections. Additionally, while the present study theorized that the dopamine and CRF projections from the VTA to the NAcc would be responsible for behavioral changes and sex differences, perhaps the VTA should be further examined for its role in the relationship. The
results of the sex difference comparisons are an initial step for better understanding sex
differences in stress-dopamine systems. However, much more work is needed in the future to
expand upon this information, including alternative approaches that could be more sensitive
measures of dopamine, measurements of stress and sex hormones, and examining other brain
regions of interest considering the results from the current study.

Future Research and Alternative Studies

The current study utilized the FR5/chow choice procedure, which was selected because
of its ability to produce stable rates of responding in both males and females. One aspect to focus
on with future studies is using a different paradigm to assess motivation. A commonly used
schedule of reinforcement for motivational studies is the progressive ratio (PR) schedule. Under
this schedule, the ratio requirement to obtain a reinforcer is increased systematically after a
reinforcer has been received (Killeen et al., 2009). Although it would be possible to develop a
PR schedule that increased by time so that it would be progressive intervals, most studies utilize
an escalating ratio requirement instead due to the reliable rates of responding and quick training.
Since the requirement to obtain a reinforcer is increased with a PR schedule, it allows
experimenters to assess when the ratio requirement becomes too high for the animal, a “break
point” which provides an approach to study motivation (Hodos, 1961). To provide a more
sensitive measure to effort, future studies could examine the impact of acute stressors and
manipulations of CRF with a PR schedule. Specifically, a PR schedule would provide more
variables that could be assessed in order to better understand the patterns that were seen in the
current study through the use of a FR5 schedule, such as individual differences and measures of
break point. In a previously mentioned study, Wanat and colleagues (2013) utilized a progressive
ratio task to examine whether CRF antagonists blocked the effect of acute stress on motivation to
work for food reward and got promising results. However, no studies have used the PR schedule with a choice component to investigate whether sex differences mediate the relationship between stress and motivation. While the overall premise of PR schedules is similar to FR5 schedules, PR schedules allow for individual differences to be examined more closely (Errante et al., 2021). Because of this, this schedule may be beneficial considering the current results, as there were some sex differences present, particularly during training. While switching the ratio schedule may or may not change the results, it would help for a closer examination of the performance of each animal and would supply more measures, such as breakpoint, so that there could be a better understanding of small changes that may be taking place in response to stress, which could be sex-specific.

Initially, this project hypothesized that the theory behind the relationship between stress and motivation was that stress leads an elevation in CRF that ultimately impacts dopamine that is the final pathway to behavior. While the results from the current study do not entirely rule out this theory, it is possible that other factors must be considered given the results from the present study. Elevating CRF did not change dopamine content in the NAcc (Experiment 3) nor did it lead to any behavioral changes (Experiment 2). While previous research has pointed to changes in CRF after restraint (Wanat et al., 2013) as well as changes in dopamine (Puglisi-Allegra et al., 1991), the current study found behavioral changes that may indicate support for these findings. Additionally, administration of the CRF antagonist antalarmin did not attenuate the reduction in motivated behavior following restraint stress in the current study. It is possible that the doses of CRF and antalarmin were not high enough to see the expected behavioral effects and thus, future studies should pursue a dose-response curve for both CRF and antalarmin. However, it is also possible that the overarching theory is inaccurate. Specifically, perhaps stress acts more
directly on dopamine, which then leads to more long-term effects on the HPA axis and CRF.

Microdialysis studies have pointed to direct consequences of stress on dopamine and its metabolites in the nucleus accumbens and prefrontal cortex (Holly & Miczek, 2016). This suggests that perhaps the elevation in dopamine is influencing the CRF system, not the other way around, or perhaps both, influence each other through complex interconnections (Kelly and Fudge, 2018). Taken together, each of these considerations supports that future studies should elucidate the connection between stress, dopamine and CRF, as it would help to inform literature on various psychological disorders and treatment methods.

Overall, the present series of experiments sought to examine the relationship between stress, motivation, and dopamine in both male and female rodents. Through the use of the FR5 chow choice procedures, it was found that yohimbine and 60 minutes of restraint stress were able to reduce performance in this motivational task as measured by total number of lever presses, with sex differences seen across some of the stressors. Dopamine was measured in the NAcc after exposure to each of the acute stressors, which showed no significant changes after stress exposure. Although some of these results were not what was expected initially, all of the results will help to inform the literature on the impact of stress on motivated behavior and dopamine in an effort to inform treatment methods in clinical populations.
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Nowend, K. L., Arizzi, M., Carlson, B. B., & Salamone, J. D. (2001). D1 or D2 antagonism in nucleus accumbens core or dorsomedial shell suppresses lever pressing for food but leads to compensatory increases in chow consumption. *Pharmacology Biochemistry and Behavior, 69*(3-4), 373-382.


## APPENDIX

### Experiment 1 Key Findings

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### Experiment 2 Key Findings

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<td>body weight (covariate)</td>
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<td>0.706</td>
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<td>sex</td>
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<td>1.762</td>
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<td>chow</td>
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<tr>
<td>chow x sex</td>
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<td>1.276</td>
<td>0.283</td>
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**Testing**

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