

INTERACTIONS BETWEEN  
STRESS RESPONSES AND SOCIAL BONDING  
IN PRAIRIE VOLES

By

STEPHANIE MCGLOTHLIN

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Rogers State University  
Claremore, Oklahoma  
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Dissertation Approved:

Tom Curtis

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Dissertation Adviser

Kathleen Curtis

---

Bruce Benjamin

---

Jennifer Volberding

---

Name: STEPHANIE MCGLOTHLIN

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Abstract: Stress is a significant factor affecting health: chronic stress is correlated with a number of diseases and disorders. Individuals in positive social relationships (pair bonds, marriage etc.) are typically healthier than those who are not. This study examines the interactions between stress and social relationships in a monogamous rodent, the prairie vole. The effects of stress on social bonding is sexually dimorphic in prairie voles: exposure to a stressor increases the likelihood of forming a monogamous “pair bond” in males, but decreases the likelihood in females. To determine whether the positive effects of stress on pair bonding in males were due to female preferences, this project examined the effect of acute stress in males on mate choice by females. It was hypothesized that acute stress in males would affect female mate choice. The goals of this project were reciprocal: to examine the effect of stress in males on mate choice by females, and then to examine the effects of pair bond formation on stress hormone levels across time and on stress behaviors. For this second goal, it was hypothesized that pair bond formation would affect stress behaviors and hormone levels across time, and predicted that stress would positively influence these measures. To carry out these goals, a new stress paradigm was designed and females were exposed to a mate choice test, during which they could spend time with a male exposed to a stressor, or a non-stressed, control male. For the second goal, fecal samples were taken at various time points across a 14-day period following pairing to measure fecal corticosterone. Males were also exposed to two behavioral tests for anxiety. The results of this study supported the first hypothesis: acute stress in males does influence mate choice by females. Stress does not positively affect mate choice, however: acute stress in males appears to negatively influence female mate choice. The results of this project partially support the second hypothesis: pair bond formation appears to influence stress hormone levels over time, but not anxiety-like behaviors.

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## CHAPTER I

### INTRODUCTION

Social relationships can have important effects on health. For example, individuals with a greater number of social relationships (stemming from relationships with family, friends, coworkers, or fellow members of a religious group) have a lower risk of mortality than individuals with fewer relationships (House et. al, 1988). Likewise, it is now known that positive relationships are associated with the prevention of different disease states: increased levels of social support in individuals are correlated with a lower risk of cardiovascular disease, cancer and death from infectious diseases (Uchino, 2006). Therefore, it is important to examine the interactions between social relationships and factors known to affect health.

Among the various adult relationships, marriage in particular has been shown to have health benefits. Married individuals enjoy lower rates of morbidity and mortality (Holt-Lunstad, 2008). Married individuals also have a lower risk for many diseases. Those who are not married have a higher incidence not only of cardiovascular disease and cancer, but also of pneumonia, influenza and other health issues (Robles & Kiecolt-Glaser, 2003). Different theories have been proposed to explain this phenomenon: among these are the social selection explanation and the social causation explanation. The social selection explanation proposes that people in

committed relationships are healthier simply because individuals already in good health are more likely to enter into such relationships in the first place. The social causation explanation, on the other hand, proposes that the relationship itself can provide different health benefits through various mechanisms (Stack, 1998; Shapiro & Keyes, 2008). While these theories are well-established, the exact causes of the benefits of marriage require further study.

Stress is one factor that can have a significant influence on health. The term “stress” is ambiguous, though the stress response is frequently defined as the body’s attempt to adapt to a perceived threat. Acute, or short-term, stress responses are important for health, as they prepare the body to respond to threats through pathways involving the nervous, cardiovascular, immune and metabolic systems. Chronic, or long term, stress can lead to various health problems through the dysregulation of these systems (McEwen, 2008). Individuals suffering from chronic stress in particular have a higher risk of developing high blood pressure, heart disease and other chronic health issues (Thoits, 2010).

There appear to be interactions between stress and social relationships. Stress, particularly chronic stress, can have a negative effect on relationships. One factor known to be affected by stress in social bonding is mate choice. Chronic stress can negatively influence sexual selection in many species: females tend to prefer males that have traits indicative of good health, which can be negatively impacted through long-term exposure to elevated stress hormones (Husak & Moore, 2008). Stress can also negatively affect the health of relationships, including marriage: research has shown a correlation between increased stress levels and decreased relationship satisfaction. Increased stress associated with the problems of everyday life has been found to be one of the most significant indicators of divorce, and current research is focused on studying how different types of stress can interrelate to accelerate marital decline (Bodenmann et. al, 2007). Unhealthy relationships can also be a significant source of stress, and those in unhappy marriages suffer from lower levels of health and well-being than individuals who are unmarried (Robles & Kiecolt-Glaser, 2003).

On the other hand, social relationships, including marriage, can protect against many of the negative influences of stress. One theory, the “social buffering hypothesis,” suggests that support from different relationships can act as a “buffer” in individuals to protect them from the negative consequences that stress can have on health. Various explanations have been proposed to describe how relationships can buffer stress: social support may provide resources that help a person manage difficult situations, may decrease the stress response by reducing the perceived significance of a stressful situation, or may directly influence physiological processes and systems (Cohen & Wills, 1985). The exact mechanisms behind the social buffering theory and how social relationships can attenuate the negative effects of stress require further examination.

This project focused on using an animal model to study the interactions between stress and social relationships. The prairie vole is a highly social rodent capable of forming monogamous “pair bonds” with a single partner (Carter et. al, 1995). Due to their social nature, prairie voles have been used in research as an animal model for studies in social bonding. The effect of stress on social bonds has previously been examined in this species: exposure to corticotropin-releasing factor (CRF) has been found to facilitate the formation of social preferences in male prairie voles (Devries et. al, 2002). Interestingly, the effects of an acute stressor, or injection of the stress hormone corticosterone, are sexually dimorphic in this species: males exposed to an acute stressor or injected with corticosterone are more likely to form a robust “partner preference” for a specific female, the first step in the facilitation of a pair bond, while females are less likely to form such a preference (Devries et. al, 1996). The mechanisms behind the effects of stress on pair bonding are not fully understood, and require further analysis.

For this project, a series of experiments were conducted to study the interactions between stress and social bonding, two factors involved in regulating health. The experiments in this project were reciprocal: first, a prairie vole model was used to study the effects of acute stress in males on mate choice by females, examining whether stress has an effect on sexual selection in this species.

Second, subjects were tested to determine whether the formation of a monogamous pair bond could affect stress responses. It was hypothesized that acute stress in males could affect mate choice by females, with the prediction that acute stress would positively influence female choice, due to previous research indicating that acute stress would increase the likelihood of partner preference formation in males (Devries et. al, 1996). It was also hypothesized that pair bond formation would influence stress hormone levels across time, as well as behaviors indicative of anxiety in common behavioral tests, with the prediction that the formation of a pair bond would have a positive effect on both of these measures for stress.

## CHAPTER II

### LITERATURE REVIEW

Prairie voles are one of only about 3% of mammalian species that form monogamous bonds between individuals in a mated pair. Due to their social nature, prairie voles can be used in the laboratory to study social bonding (Carter et al., 1990). A number of factors influence social bonding in this species, including stress and the stress hormone corticosterone (Devries et al., 1996). Another factor influencing social bonding in many species is mate choice. “Mate Choice” is the inclination of members of one sex to choose mates due to a bias toward certain traits in the opposite sex (Kokko et al., 2003). Female mate choice can be influenced by a variety of factors in different species, including a male’s genes, physical characteristics and mating behavior (Jennions & Petrie, 2007; Byers & Kroodsma, 2009; Williams, 2001; Husak & Moore, 2008). Stress can also be a factor in mate choice: chronic stress in particular negatively influences mate choice in many species (Kokko et al., 2003). While stress can have a negative effect on social bonding by influencing mate choice, prior social bonds can potentially attenuate the stress response. Through various mechanisms, social support can decrease stress and provide a positive influence on various health outcomes (Cohen & Wills, 1985; Kikusui et al., 2006).

## **1. The Prairie Vole as an Animal Model**

### **Prairie Voles in the Laboratory**

The prairie vole is a highly social species, exhibiting the characteristics of a monogamous mating system (Carter et al., 1995). Prairie voles are useful laboratory animals for other reasons as well: they successfully breed in captivity, producing litters consisting of 1-8 pups. The gestation period for prairie voles is only 21 days, and they can be weaned at postnatal day 21 (Nadeau, 1985). Finally, based on the animal's size and geographic distribution, prairie voles are easily obtainable and relatively inexpensive to care for in a laboratory setting (Carter et al., 1995). For these reasons, the prairie vole is an excellent animal model for use to study social bonding.

### **Natural History**

The majority of vole species live in grassland habitats (Getz et al., 1987). Prairie voles, specifically, are found in the grasslands located throughout central North America (McGraw & Young, 2010). They are thought to have evolved in tallgrass prairies, environments that are relatively low in food resources. In this type of habitat, prairie vole populations tend to be low density, with females widely dispersed (Carter et al., 1995). Prairie vole populations typically consist of communal groups and single, "wandering," animals. Perhaps due to harsh conditions, prairie voles exhibit alloparental care with both sexes involved in caring for offspring in communal groups. These groups are comprised of a male-female pair and their offspring; up to 70% of juvenile voles may continue to live in the parental nest instead of dispersing (Carter et al., 1995; McGraw & Young, 2010). Communal groups live in underground nests, and prairie voles frequently create tunnels through vegetation and dead litter aboveground. The home range of a prairie vole can be up to 300 m<sup>2</sup> (Getz et al., 1987).

Prairie voles are common prey animals. Potential predators include birds of prey, small mustelids, feral cats and snakes (Tamarin, 1985). Thus, the size of communal groups varies by

season due to levels of predation: nests typically have a higher number of voles in the winter than in spring and summer, when predators increase. A major cause of juvenile mortality in prairie voles is snake predation: experiments have shown that when snakes are prevented from attacking nests, communal groups maintained the same size in the summer as they did in the winter (Getz et al., 1990; Norrdahl & Korpimaki, 1998).

### **Social Behavior**

As noted, prairie voles form monogamous bonds with their respective mates (McGraw & Young, 2010). The first indication that prairie voles were monogamous came from population studies: animals collected from traps were frequently caught as male/female pairs. This finding, as well as laboratory observations of males caring for their young, led to the idea that prairie voles have a monogamous mating system. Further observations showed that prairie voles displayed many of the characteristics of monogamy: cooperative nest building, defense and grooming of the young, food acquisition, retrieval of young, and territorialism by both sexes (Thomas & Birney, 1979). Due to these characteristics, the prairie vole is an ideal candidate as an animal model for monogamy.

### **Pair-Bonding Regulation**

There are multiple factors that affect pair bonding in this species. Mating is important for the development of a pair bond, and female prairie voles will not go into estrus until they are exposed to a pheromone in the urine of an unfamiliar male (Carter et al., 1980). While short-term “partner preferences” may develop following mating and cohabitation, prairie voles do not maintain long-lasting pair bonds unless the female becomes pregnant within two to three days following exposure to a novel male (Curtis, 2010). Pair bond formation is also heavily regulated by the neuroendocrine system. A number of neurochemicals are involved in the formation and maintenance of a pair bond in this species. These include the neuropeptides oxytocin and arginine

vasopressin, the neurotransmitter dopamine, and the steroid hormone corticosterone (Carter et al., 1995; Young & Wang, 2004). The following is a short description detailing how each neurochemical facilitates the development of a monogamous pair-bond.

### *Oxytocin and Vasopressin*

Initial studies on the neurobiology of pair-bonding examined the neuropeptides arginine vasopressin (AVP) and oxytocin (OT). These two hormones were examined because of their known involvement in different social behaviors, such as mating behavior, aggression, and maternal bonding (Wang et al, 2010). Infusion of OT into the cerebral ventricles facilitates pair-bonding in females, while infusion of AVP facilitates pair-bonding in males. After receiving an OT receptor (OTR) antagonist, pair-bond formation is inhibited in females, while pair-bond formation is inhibited in males following the administration of an AVP receptor 1a (V1aR) antagonist. While both OT and AVP are important for pair bond formation, it appears that OT is more important for the development of a pair-bond in females, while AVP is more important for males (Young & Wang, 2004).

Several brain regions have been implicated in the involvement of OT and AVP in regards to pair-bond formation. The nucleus accumbens is particularly important for pair bonding (Wang & Liu, 2003). Prairie voles have a higher density of OTRs in the nucleus accumbens and ventral pallidum compared to a promiscuous vole species (Young et al., 2005). Likewise, prairie voles have higher densities of V1a receptors in the ventral pallidum, medial amygdala and mediodorsal thalamus compared to promiscuous voles. The nucleus accumbens, prefrontal cortex, lateral septum and ventral pallidum have been implicated in pair-bond formation (Young & Wang, 2004).



## *Dopamine*

Dopamine (DA) involvement in pair-bonding has been supported by different pharmacological studies: the administration of the DA agonist apomorphine facilitated partner preferences in female prairie voles, while exposure to the antagonist haloperidol inhibited partner preference formation (Young & Wang, 2004). Experiments utilizing male prairie voles yielded similar results. The nucleus accumbens has been implicated as an important region for the dopaminergic regulation of pair bonding: administration of haloperidol to this region inhibited partner preferences, while exposure to apomorphine did the opposite. Importantly, the nucleus accumbens contains a high number of dopamine receptors, particularly in the shell (Aragona et al., 2003).

There are two different types of dopamine receptors: D1 and D2-type receptors. A variety of evidence suggests that the D2-type receptors are involved in pair bond formation. Studies with female prairie voles found that upon administration of the D2-type agonist quinpirole, but not the D1-type agonist SKF 38393, subjects formed partner preferences (Aragona et al., 2005). It has been suggested that D1-type receptors may prevent pair bond formation. This suggestion is supported by the finding that pair bonded males have increased D1-type, but not D2-type receptors, in the nucleus accumbens. This increase in D1-type receptors may inhibit the development of new pair bonds and thus help maintain the existing pair bond (Aragona & Wang, 2004).

An interaction between dopamine and oxytocin is necessary for pair bond formation. Inhibition of OT receptors blocked partner preferences stimulated by a D2-type agonist in the nucleus accumbens. Likewise, inhibition of D2-type receptors prevented partner preferences induced by an OT-agonist. These findings suggest that the concurrent stimulation of both OT and

D2-type receptors in the nucleus accumbens is necessary for pair bond development, at least in female prairie voles (Liu & Wang, 2003; Young & Wang, 2004).

### *CRF and Corticosterone*

Corticosterone and corticotropin-releasing factor (CRF) have also been implicated in pair-bond development. The effect of corticosterone is sexually dimorphic in prairie voles: male prairie voles exposed to a three-minute forced swim test or injected with exogenous corticosterone were more likely to form partner preferences, while the opposite effect was seen in female prairie voles. A potential explanation for this phenomenon is that males that are stressed from overcrowding in the natal nest may be more likely to disperse and find mates. However, the exact causes of this phenomenon are not yet well understood (Devries et al., 1996). Exposure to corticotropin-releasing factor (CRF) can also facilitate social preferences in male prairie voles: following a three-hour cohabitation period with an opposite-sex conspecific, males treated with 0.1 or 1.0 ng CRF spent a significantly greater amount of time in physical contact with the familiar female than did other males in the experiment. These results indicate that the hypothalamic-pituitary-adrenal (HPA) axis is involved in pair bonding (Devries et al., 2002).

## **2. Mate Choice**

### **Mate Choice as a Means of Reproduction and Adaptation**

In his book, *The Descent of Man*, Charles Darwin described his theory of sexual selection and its potential effect on evolution. Darwin listed two primary methods of sexual selection: the first relying on competition between members of the same sex for access to mates, and the second relying on female mating preferences, which includes competition between males to appeal to females (Brock & McAuliffe, 2009). This latter method, commonly referred to as selection by mate choice, is seen throughout different species of the animal kingdom. “Mate choice” is defined as the inclination of individuals of one sex to breed nonrandomly due to a bias toward certain

traits in individuals of the opposite sex (Kokko et al., 2003). While mate choices can be made by either sex, females are often more motivated by preferences than males. The reason for this may be due to the higher investment in offspring and reproduction females make: developing eggs/fetuses and caring for young requires greater energy investment (Krasnec et al., 2012).

Three pressures can potentially drive the development of preferences in different species: preferences could be made that directly improve the chances for female survival or fertility, could be based on pleiotropic effects of natural selection in conditions away from mate choice (foraging, predator avoidance, etc.), or could be based indirectly on traits that increase the fitness of offspring (Jennions & Petrie, 1997). Offspring fitness is affected by parental genes: females may prefer males with traits indicating quality genes through good body condition (ornamentation, etc.), or may prefer males that are genetically dissimilar to themselves, thus promoting genetic variability in offspring (Mays & Hill, 2004).

The pressures for mate choice listed are a means of natural selection. When a female prefers a male that increases her chances for survival and reproductive success, this is an example of direct selection. Direct selection leads to a preference for males that seem fertile, offer a high number of resources, provide parental care or decrease the female's reproductive costs in other ways. The benefits for this model are straightforward: females will "see" a direct benefit to their own fertility, and a relief to reproductive costs. For this reason, this model is the least controversial method of mate choice (Kokko et al., 2003).

Some species may also rely on indirect benefits, leading to an increase in the fitness of offspring (Kotiaho & Puurtinen, 2007). As described in the 'Fisherian runaway process,' females that choose fitter males will produce offspring that inherit traits leading to better fitness and the resulting mating preference. It has been hypothesized that females should choose males that are more robust and have greater longevity, thus, traits that are considered "attractive" could be used

as indicators of vitality. Fisher's original model focused on the idea that mating preferences were due to a genetic correlation between whatever traits the females preferred and the fitness of later offspring (Kokko et. al, 2003). The Fisherian runaway process is a self-reinforcing method that can potentially lead to the exaggeration of a particular (frequently ornamental) male characteristic and the associated female preference for said characteristic, as a representation of health and fitness (Takahasi, 1997).

### **Factors Influencing Mate Choice**

As stated, females likely make mate choices based on perceived survival benefits and the potential for improved fitness of offspring (Jennions & Petrie, 1997). However, a female's choice may be limited by several constraints: time, mobility and memory. A female's choice is limited by the number of males she encounters during her fertile period, the males she encounters while moving through individual territory, and by her own memory of the males she has previously encountered (Janetos, 1980). Due to limitations placed upon the female, and the effects mate choice can have on offspring fitness, it is important to recognize the factors that can influence female mate preferences. Female mate choice can be influenced by factors such as genetics, physical appearance, behavior and stress (Kokko et al., 2003; Jennions & Petrie, 2007; Byers & Kroodsma, 2009; Williams, 2001; Husak & Moore, 2008).

#### *Genetics*

As discussed, females may choose mates that would enhance the genetic fitness of offspring (Kokko et al., 2003). Certain species, for example, use "indicator traits" (antlers on deer, plumage of birds, etc.) as signs of male quality that females can use to form preferences based on genetics (Mays & Hill, 2004). While females often choose males with physical characteristics that represent quality genes, the influence of genetics on mate choice is also more complicated. The main problem with mate choice based on heritability is that if females only

chose males based on preferences for certain traits, then variability for these traits would disappear. Another theory posits that females not only choose males with superior genes, but also prefer males with which they are genetically compatible. In this theory, the specific combination of male and female genotypes determines offspring fitness, so the male with the “best” genes for one female may not have the best genes for another (Tregenza & Wedell, 2000).

Females of different species may choose mates based on either quality genes or compatibility. There is evidence supporting female preferences in certain species for traits indicative of good body condition and quality genes (ornamentation, etc.); however, there is also evidence to support preferences based on genetic compatibility (Mays & Hill, 2004). Evidence for preferences based on genetic compatibility can be seen in the low occurrence of incest throughout many species. There is a potential genetic disadvantage to inbreeding, as it could lead to an increased number of offspring homozygous for harmful alleles. Incest avoidance causes the incidence of deleterious alleles to be lowered by selection, and thus only a minimal number of individuals in a population will be homozygous for the affected allele (Tregenza & Wedell, 2000).

Mate choice based on genetic compatibility has also been studied through the examination of the major histocompatibility complex (MHC), a group of genes that produce proteins important for regulating the immune system. MHC molecules work to present viral peptides (known as antigens) to T cells in order to induce an immune response (Milinski, 2006). MHC encodes for proteins involved in antigen-presentation to T-cells, so organisms with differences in these complexes show differences in their resistance to foreign invaders and susceptibility to autoimmune disorders. MHC diversity is important, as individuals with a heterozygous MHC benefit from being able to bind to double the amount of antigens. Females that have mated with males with a similar MHC have an increased rate of miscarriages in humans and other primates (Tregenza and Wedell, 2000).

Mate choice based on MHC compatibility has been documented in several mammals including mice, rats and humans. The importance of MHC genes can be illustrated by the fact that a group of similar genes most likely exists in all vertebrate species. Early studies in both inbred and wild-type mice showed that mate choice was biased toward “non-self” MHC types. Studies have continually shown MHC-disassortative mating in both sexes, with homozygotes showing the greatest preferences (Tregenza and Wedell, 2000; Yamazaki & Beauchamp, 2007). Human females also prefer males with different MHC types than their own: humans as well as mice appear to make their preferences for certain individuals with MHC compatibility based on detecting different odors (Wedekind et al., 1995). It has been proposed that preferring individuals with different MHC types than one’s own is also a mechanism for inbreeding avoidance: individuals are more likely to select against individuals with MHC types similar to their own, thus avoiding mating with related individuals (Yamazaki & Beauchamp, 2007).

### *Physical Characteristics*

Preferences can only be formed if there is sufficient diversity among male phenotypes to allow the female to perceive differences (Jennions & Petrie, 1997). Males in many species, particularly birds, rely on ornamentation to attract mates. “Ornaments” can be prominent plumage, combs, brightly-colored gapes, wattles or any other exaggerated physical trait that can be attractive to the directed female. Well-developed ornaments have been shown to be useful indicators for quality in male-male competition and mate choice (Sun et al., 2013). The use of such ornaments may employ the concept of “honest signaling,” whereby a signal (such as ornamentation) will correctly represent the quality of the organism. In this concept, signals such as ornamentation or other displays will require a significant amount of energy to produce, being costly to the organism. Thus, a signal such as ornamentation would indicate that an organism’s is healthy enough to survive, even with the added “cost” of the signal (Dawkins & Guilford, 1991).

Studies have revealed that females that chose males with exaggerated ornaments benefitted from factors such as increased paternal care for offspring, improved habitats, fewer parasitic infections, and increased reproductive opportunity for male offspring (Sun et al., 2013). Ornaments are thought to have evolved either due to sexual selection for increased reproductive success, or by social competition for higher chances of offspring survival. Ornaments may have developed as signals for mate quality (existing as indicators for quality genes or overall health) in mate choice as discussed above, or of improved ability to compete with males for females (Candolin & Tukiainen, 2015).

### *Behavior*

Courtship behavior is important for mate choice in a variety of species. In many species of birds, courtship may include song as well as a “dance,” a pattern of actions that include certain postures, “puffing up” of plumage and ritualized movements. Females may choose males based upon the quality of their birdsong or the pattern of their dances, although the mechanisms behind this choice are not well understood (Byers & Kroodsma, 2009; Williams, 2001). Male field crickets are also known to produce a “calling song” that affects female mate choice, with females being able to differentiate between males based on factors such as chirp rate, chirp duration, the rate between chirps, and the amplitude of chirps (Wagner & Reiser, 2000). Courtship behavior also exists in certain species of fish: guppies are known to form preferences based on the strength and time of male courtship displays, as well as color patterns found in males (Kodric-Brown, 1993).

### *Stress*

Stress-particularly chronic stress-has also been found to be a factor in female mate choice. Elevated levels of baseline glucocorticoids are interpreted as representing a diminished body condition and decreased fitness (Bonier et al., 2009). As a male’s health and overall

condition is negatively correlated to baseline glucocorticoid levels, females typically prefer males with lower plasma levels in order to breed with mates of better quality. Glucocorticoids are secreted into the bloodstream, and receptors for them are abundant in tissues throughout the body, and produce a range of effects. Thus, many of the traits a female could use for mate choice may be affected by glucocorticoids and stress (Husak & Moore, 2008).

Evidence from different animal species has shown that females routinely select against males with elevated baseline glucocorticoids. Female zebra finches, for example, have been found to prefer males with low circulating levels of plasma corticosterone as opposed to males with high circulating levels of the glucocorticoid (Roberts et al., 2007). Stress can negatively impact the physical and behavioral characteristics females often use to determine preferences: an elevation in circulating glucocorticoids can negatively impact song quality in zebra finches, vocalizations in different amphibians, and ornamentation in birds (Husak & Moore, 2008).

### **Mate Choice in the Prairie Vole**

A number of studies have examined mate choice by prairie voles. Several factors may influence a female prairie vole's preferences. Getz and colleagues (2005) reported that females usually avoided mating with family members, instead preferring unrelated individuals. Females do not usually engage in anogenital sniffing with familiar males, a necessary act for mating, as pheromones from male urine are required to induce estrus. As such, the chemosignals necessary to induce estrus and mating are usually acquired from unfamiliar males.

Once they have mated, paired females will prefer the male they have previously mated and cohabitated with. This finding led to the conclusion that copulation was necessary for the formation of a preference toward a specific animal (Shapiro et al., 1986). Long-term preferences in prairie voles only occur if mating is successful. A long-lasting, monogamous "pair bond" only



develops between a pair if the female becomes pregnant within two to three days after meeting a novel male (Curtis, 2010).

Other factors can affect mate choice in this species. Female prairie voles prefer males with a longer anogenital distance, which has been correlated with testes size and seminal vesicle size. Thus, females may prefer a longer anogenital distance because it could indicate higher fertility in males (Ophir & delBarco-Trillo, 2007). A male's litter composition also affects mate choice in females, with females avoiding males born from all-male litters. In contrast, females show no differences in preference or avoidance for males born in mixed-sex or single pup litters (Curtis, 2010). Social hierarchy may also play a role in female mate choice. One study examined female preferences toward "dominant" and "subordinate" males, with "dominant" males characterized as animals that initiated attacks and spent the majority of time alone when in a cage with a novel male. Female voles consistently preferred dominant over subordinate males (Shapiro & Dewsbury, 1986).

Preferences between animals utilizing "partner preference" tests have often been studied in prairie voles. Such tests have previously been used to test a female's preference for one male over another (Carter et al., 1995; Shapiro & Dewsbury, 1986). While mate choice has been examined in this species before, the effects of preferences on mating and reproduction requires further study, particularly regarding the effects of different factors, such as male stress, on mate choice in this species.

### **3. The Interaction between Stress, Social Relationships and Health**

#### **Biological Pathways for Stress**

The stress response is an important mechanism for self-protection, as it orchestrates an organism's reaction to a perceived threat. There are two major pathways regulating the stress response in mammals: the sympathetic nervous system (SNS) in the "fight or flight" response,

and the hypothalamic-pituitary-adrenal (HPA) axis. Both pathways may be activated in response to a threat, and work to mobilize the resources necessary for an organism to react (Vale & Smith, 2006).

The fight-or-flight response refers to the physiological and behavioral responses of an organism to a potential threat. When exposed to a predator, for instance, an organism must prepare to fight back or flee from the situation. This response is characterized by activation of the sympathetic nervous system (SNS). The activated SNS will then stimulate the adrenal medulla to release the catecholamines norepinephrine and epinephrine into the bloodstream (Taylor et al., 2000). The actions of these molecules on various receptors leads to a coordination of actions typical of the fight-or-flight response: increased blood pressure, increased blood glucose, a rise in metabolic rate and an increase in alertness. These actions prepare the body to mobilize against a threat (Costanzo, 2010).

The hypothalamic-pituitary-adrenal (HPA) axis consists of the paraventricular nucleus (PVN) of the hypothalamus, the anterior pituitary gland, and the adrenal cortex. Upon the perception of a threat, neurons in the PVN produce corticotropin-releasing factor (CRF). CRF is released through the hypophysial portal to the anterior lobe of the pituitary gland, stimulating the release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH stimulates the adrenal cortex to secrete glucocorticoids into the general circulation (Vale & Smith, 2006). Glucocorticoids in the bloodstream act to promote gluconeogenesis. Allowing for an increase in blood glucose, and a resulting increase in energy to act against a threat. The HPA axis is regulated through a negative feedback loop: the secretion of glucocorticoids from the adrenal cortex acts negatively on the hypothalamus, decreasing ACTH and CRF secretion (Silverthorn, 2016).

The stress response is beneficial when reacting to acute stress. Changes induced by the SNS during the fight-or-flight response function to prime organisms for physical confrontation or even to react to severe conditions such as acute hemodynamic collapse or respiratory compromise. SNS activation in these situations have beneficial effects on an organism's survival and later fitness (Curtis & O'Keefe, 2002). HPA axis activation is also beneficial for acute stress. Increased production of glucocorticoids induces gluconeogenesis, allowing for increased energy to confront potential threats (Costanzo, 2010). While these systems are important for survival in situations of acute stress, however, they may be detrimental if activated for longer periods of time.

### **Negative Effects of Stress on Health**

There are three stages for a physiological reaction to a stressor: the alarm, resistance and exhaustion stages. During the exhaustion stage, when there is a decrease in the body's ability to resist stress, there is an increased risk of high blood pressure, heart disease and other chronic health issues (Thoits, 2010). Chronic stress has been correlated with other issues in animal models, including hypertrophy of the adrenal gland, atrophy of lymphatic organs, and stomach ulcers. Further data show that short-term, intense episodic or chronic exposure to different stressors can have similar effects in humans (Guilliams & Edwards, 2010).

With chronic stress, both the SNS and the HPA axis contribute to negative health effects. Chronic sympathetic activation increases in the production and storage of the catecholamines norepinephrine and epinephrine, and leads to elevated levels of these hormones in the blood (McCarty et. al, 1988). Chronic activation of the SNS increases the workload of the cardiovascular system and leads to heart problems such as coronary spasms, dysrhythmias and hypertension (Curtis & O'Keefe, 2002). Chronic sympathetic activation is correlated with other issues as well, including obesity, sleep apnea, kidney disease and heart failure (Malpas, 2010).

Chronic HPA axis activation can lead to periods with increased glucocorticoid levels that are not decreased by negative feedback inhibition. Glucocorticoids have immunosuppressive actions through multiple mechanisms, so long-term increases can increase the risk of disease and infection (Newton, 2000; Guilliams & Edwards, 2010). Glucocorticoids are also inhibitory to the secretion of growth hormone, thyroid-stimulating hormone and gonadotropins (Tsigos & Chrousos, 2002). The inhibition of these hormones negatively affects growth and reproduction. Elevated glucocorticoid levels are correlated with delayed puberty, anovulation and miscarriage in women, and decreased testosterone levels and impaired spermatogenesis in men. Inhibition of thyroid-stimulating also negatively affects thyroid function, leading to problems with growth and metabolism. Chronic activation of the HPA axis has been correlated with a range of other health issues, including mental disorders, such as obsessive-compulsive disorder, panic disorder and clinical depression (Guilliams & Edwards, 2010).

In some cases, chronic stress may lead to an adaptation of the HPA axis where glucocorticoid production is reduced. This adaptation may be caused by reduced glucocorticoid signaling and changes in the negative feedback loop. This hypoactivation of the HPA axis can cause a variety of health issues as well. Individuals with seasonal depression and chronic fatigue syndrome display hypoactivation of the HPA axis. Other disorders correlated with reduced HPA axis activation include hypothyroidism, fibromyalgia and rheumatoid arthritis (Tsigos & Chrousos, 2002; Guilliams & Edwards, 2010).

### **The Effect of Social Relationships on Health**

“Social support” usually refers to the quantity and quality of a person’s social relationships. References to social support typically include the framework of a person’s ties (group memberships, family members, etc.) and the direct benefits they create, such as emotional support. Since the 1970s, studies have shown that people with less social support had higher

mortality rates. Even when controlling for the effects of initial health, studies indicate that those with strong social networks have better health outcomes. High levels of social support are correlated with lower risk for cardiovascular disease, cancer and infectious disease mortality (Uchino, 2006).

There are three potential mechanisms by which social relationships can impact health: behavioral, psychosocial, and physiological. Studies have shown that relationships can affect our health habits (diet, physical activity, etc.). Overall involvement with organizations such as church as well as family and friends have been correlated with more positive health behaviors. Social relationships can influence habits pertaining to an individual's health by imparting a sense of responsibility and care for others that lead one to behave in ways that protect the health of others and themselves. Relationships provide for the opportunity to share information and create norms that affect health habits.

In terms of psychosocial benefits, social ties may be helpful to mental health and, in turn, physical health as well. Involvement in positive social relationships may indirectly influence mental health by reducing stress levels, or by providing meaning and purpose to life. Social support may increase a person's feelings of personal control, which can have positive effects on health habits, as well as mental and physical health. There are also physiological effects of social support on health. Social support correlates to benefits on the immune, endocrine, and cardiovascular systems. Childhoods characterized by strong support systems are correlated with the development of healthy regulatory systems, such as the immune system, autonomic nervous system, and the hypothalamic-pituitary-adrenal (HPA) axis (Umberson & Montez, 2010).

### **“The Marriage Benefit”**

The most significant relationship in the lives of many people may be the relationship they have with their spouse. For this reason, there has been a focus on the effects of marital

relationships on health. Previous studies have shown that married individuals have lower rates of morbidity and mortality than do those who are unmarried. Those who are married also report greater life satisfaction, happiness, and fewer incidences of depression (Holt-Lunstad, 2008). The effect of marital status on health is fairly consistent across studies, with marriage providing greater protection for men than women.

Just how marriage can affect health is unknown, although multiple explanations have been presented. The effects of cohabitation, financial security, and social support have all been examined, with the two latter explanations being supported by research. Married individuals enjoy greater financial security, with the median household income for married individuals being much higher than those who are not married. Even controlling for income, however, married individuals still have lower rates of mortality (Johnson et al., 2000; Robles & Kiecolt-Glaser, 2003).

Marital status may affect health in several ways, including interpersonal mediators, psychological processes, coping strategies, and physiological consequences. There is also evidence that close relationships can affect disease processes or outcomes through changes in mood and their effects on health habits. Close relationships, including marriage, affect physiology including cardiovascular, immune, and neuroendocrine function. Many of the psychological and physiological pathways thought to explain the influence on health involve the stress/social support hypothesis, which describes how stress and support in a marital relationship can affect health through different pathways. These pathways include effects on the marriage itself, on a person's thoughts or feelings, health-related and coping behaviors, as well as physiology (Robles & Kiecolt-Glaser, 2003).

## **Stress and Social Buffering**

Another theory to explain the effects of social support on health is the “social buffering hypothesis,” which primarily affects individuals undergoing different forms of stress. Stress, in this theory, occurs when an organism is confronted with a situation that is dangerous or otherwise demanding and does not have the means to enact an appropriate response (Cohen & Wills, 1985). Chronic stress has already been shown to be correlated with many health problems (Thoits, 2010; Malpas, 2010; Guilliams & Edwards, 2010). The social buffering hypothesis suggests that social support can act as a “buffer” in individuals to protect them from the negative impact of stress on health. (Cohen & Wills, 1985).

Social buffering has been displayed in many social species, including rats, guinea pigs, humans and other primates. In these animals, individuals exhibit enhanced recovery from aversive situations when they are in the presence of others. For example, a cat’s nervousness about eating decreased when watching another cat eat, goat kids were more able to manage a new environment when they were with their mother, and the fear response rats displayed in an open field decreased when they were joined by other rats. Experiments with squirrel monkeys have also demonstrated social buffering: infant monkeys display a less pronounced increase in cortisol when being separated from their mothers in the presence of conspecifics as opposed to when they are alone (Kikusui et al., 2006).

Multiple mechanisms have been proposed to explain the buffering effect of social support on stress. Social support could play two roles in alleviating stress. First, the knowledge that one has a network of individuals willing to provide necessary resources may decrease the perception of potential for harm in a given situation and increase a person’s ability to manage demanding situations. Alternatively, social support may attenuate the stress reaction (by bringing solutions to

problems or reducing the significance of a problem) or directly affect different physiological processes, including neuroendocrine processes (Cohen & Wills, 1985).

Evidence shows that social buffering can affect the HPA axis. In addition to work with squirrel monkeys showing that the presence of other monkeys can attenuate the increase in cortisol seen after exposure to a stressor, other experiments have shown that the presence of a familiar animal can decrease neuroendocrine responses to stressors. Rats also display a lower corticosterone response when introduced into a new environment when they had a partner than when they were introduced alone. The PVN appears to be responsible for the effect of social buffering on the HPA axis. Experiments in rats and sheep have shown that the PVN is less active in response to a stressor when the organism is in the presence of other animals than when alone (Kikusui et al., 2006). As the PVN is one of the first structures involved in the release of glucocorticoids and the regulation of the neuroendocrine stress response, this goes a long way in explaining the effect of social buffering on stress.

Social support appears to have its effect on the HPA axis by two different mechanisms. The first is through the production of oxytocin, which can attenuate some of the behavioral and physiological effects of stress and anxiety. Intracerebral oxytocin inhibits the activity of the HPA axis (Heinrichs et al., 2003), which may involve three different pathways: (1) peripheral oxytocin can act on the adrenal gland to decrease the production of glucocorticoids, (2) peripheral oxytocin can decrease ACTH production in the pituitary gland or (3), central oxytocin can decrease activation of CRF receptors in the PVN (Legros et al., 1987; Neumann et al., 1998; Windell et al., 1997; Kikusui et al., 2006). As oxytocin is important for social bonding, this neurohormone may be involved in mechanisms through which social support affects the stress response. The opioid system may also be involved. Social contact can cause the release of opioids, and opioids mitigate the stress response in separation-induced anxiety behavior. The opioid system has a powerful



rewarding effect, which may explain why social animals seek affiliation, resulting in a reduction of stress (Kikusui et. al, 2006).

### **Marriage, Social Buffering and Stress**

A perception of support from one's spouse has been shown to increase marital satisfaction, and one of the ways spousal support can increase marital fulfillment is by averting stress-related deterioration in the marriage (Brock & Lawrence, 2008). Social support is most effective when it is provided by those who share similar values and characteristics and have dealt with similar stressors. A spouse is usually a primary source of similarity and shared experiences, and spousal support may be prioritized over other forms. Spouses are the first individuals reached out to during moments of crisis, and during moments of severe grief, such as when a parent loses a child, stress levels are significantly reduced through spousal support (Dehle et. al, 2010). Married individuals that report high levels of marital satisfaction and support have lower ambulatory blood pressure than single individuals or unhappily married individuals (Ryan et. al, 2014).

While married relationships can provide a positive influence on stress and health, the quality of the marriage matters. In other words, social relationships, including marriage, can also be a source of stress. Poor marital quality has been correlated with immune and endocrine dysfunction, as well as mental disorders such as depression. Marital strain negatively influences health, with the impact becoming greater with age (Umberson & Montez, 2010). Marital conflict is a major source of stress, and is correlated with poorer health: individuals experiencing significant marital strain have greater psychological distress and depressive symptoms, are slower to recover from disease, have lower self-reported health and greater pain (Kikusui et. al, 2006). While individuals in reportedly happy marriages appear to benefit from the buffering of stress and

greater health, unhappily married individuals report the opposite. The quality of a marriage-and other social relationships-can potentially determine an individual's stress and overall health.

#### **4. Conclusion**

There are significant interactions between stress and social bonding. Chronic stress can negatively impact an individual's health and overall body condition, which in turn can negatively influence mate choice in different species. Social bonds can also influence stress levels: social support can decrease stress levels and may provide a buffering effect for many of the negative physiological outcomes of chronic stress. The influences of social bonding on stress and health have previously been studied in the prairie vole, a monogamous rodent. The effects of stress on social bonding has before been studied in prairie voles: males exposed to a stressor are more likely to develop a long-lasting social preference, while females are less likely to do so. However, the influences of stress on the specific factor of mate choice have not been thoroughly examined in this species. The effects of pair bonding on different stress measures in prairie voles also require further study. The goals of this project were to examine the effects of stress in males on mate choice by females, and to determine whether the formation of a monogamous bond would influence stress hormone levels across time, as well as behavioral measures of anxiety in male prairie voles.

## CHAPTER III

### MATERIALS AND METHODS

The aim of this project was to examine reciprocal interactions between stress and social bonding. To pursue this goal, two experiments were conducted: the first focused on the influence of acute stress in males on mate choice by females, and the second on the effect of pair bonding on hormonal and behavioral measures of stress. Several methods were employed to carry out these experiments. Mate choice tests were first conducted to study the influences of acute stress on social bonding. Following the mate choice tests, females were paired with one of the males they had been exposed to during the mate choice paradigm. Fecal samples were collected from the males at different time points across 14 days and tested for the stress hormone corticosterone. Finally, males were subjected to two different behavioral tests to examine the influence of pair bonding on stress responses.

## **Subjects**

Prairie voles used in this project were between 60-120 days old. Males weighed an average of 38.0 g and females an average of 42.7 g. Prior to the experiment, animals were housed with same-sex cage mates (usually siblings) following weaning at 21 days of age. Animals were given *ad libitum* access to food and water. Food consisted of Purina rabbit chow with sunflower seeds as a supplement. All animals were on a 14/10 hr light/dark cycle. All animals were mature but sexually naïve at the beginning of these experiments. All work with animals in this project was in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was conducted with the approval of the Oklahoma State University Center for Health Sciences Animal Care and Use Committee.

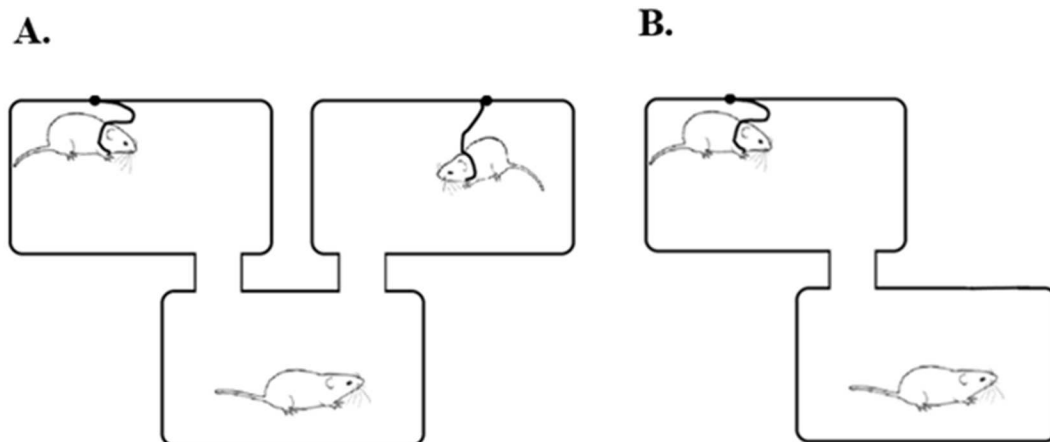
## **Mate Choice Testing**

For the mate choice test, female voles were given a choice between two males: a male that had been exposed to an acute stressor, and non-stressed control male. Males in the stressed group were first subjected to the acute stress paradigm developed in preliminary experiments (see “Methods Development” chapter): a five minute resident-intruder encounter with a mated male from our breeding colony followed by 15 minutes of sharing the same cage as the mated male, while being separated by a wire mesh barrier. The non-stressed control males in the experiment underwent no stress manipulations.

The mate choice apparatus consisted of three cages connected to each other by plastic tunnels (see Figure 1A). Each of the cages contained bedding, and animals had access to food and water *ad libitum* in each of the cages throughout the test. In one of the cages, the stressed male was tethered, so that the animal was restricted to the individual cage, unable to move throughout the apparatus. Likewise, the non-stressed male was tethered in the second cage. The female was placed in the third, empty cage, and allowed to explore the apparatus for a period of 120 minutes.

The 120-minute period was video recorded, and Noldus live tracking software was also utilized to track the subjects' movements.

In previous experiments with voles, a female was said to have a “preference” for one or the other male when she spent more time with one animal over the other (Carter et al., 1995). Our preliminary tests showed, however, that females rarely spent time with both males during the procedure. Females then were said to have made a “choice” if they spent 10 or more minutes with a specific male. Females that chose a male were paired with their preferred male for 14 days following the mate choice test. Females that did not make a choice (i.e., showed no preferences for either male) were randomly assigned to be paired with the “stressed” or “non-stressed” male.



**Figure 1: Mate choice and aversion testing.** A) The “partner preference” apparatus used in this experiment for mate choice testing. B) The modified “partner preference” apparatus used for aversion testing.

## **Aversion Testing**

Based on the results from the mate choice tests, it was questioned whether females preferred non-stressed males or had an aversion to males that had been stressed. To answer this question, a modified mate choice test was conducted in which females had the option of spending time with a male that had been stressed, or spending time in an empty cage. Results were compared with those from tests where females instead had the option of spending time with a non-stressed control male vs. an empty cage.

The aversion test consisted of a similar apparatus to that used in the mate choice test, except that it consisted of two cages connected to each other by a single tunnel, as opposed to three cages with two tunnels (Figure 1B). Prior to the test, males were exposed to the same stressor as that outlined in the mate choice experiment. Males were then tethered in one of the two cages, while the female subject was placed in the opposite, empty cage and allowed to move freely for 120 minutes. Similar to the mate choice experiment, the 120 minutes was video recorded for later review, and tracking software was used to record the animals' movements. Following testing, males and females were placed in a chamber where they were exposed to CO<sub>2</sub> gas for five to ten minutes. Following CO<sub>2</sub> exposure, subjects were immediately decapitated and their trunk blood collected for plasma hormone analysis.

## **Fecal Analysis for Corticosterone after Pairing**

Fecal samples were taken from all males prior to the mate choice test. Subjects were isolated for one hour in an empty cage during each sample collection period. Samples were then collected 24, 48 and 72 hours following the initial collection, after males and females were paired. Fecal samples were also collected on days 5, 7, 10 and 14 after pairing. Samples were stored in a -80° Celsius freezer until testing. Fecal samples were then analyzed for corticosterone using the ELISA testing protocol designed in preliminary experiments (see "Methods

Development” chapter) to assess changes in corticosterone levels during the 14 days of pairing with a female partner. Corticosterone measures were normalized by subtracting baseline (day 0) values from all other measurements across the 14-day period for each male. Following normalization, fecal corticosterone levels were compared between bonded and non-bonded males, as well as between males paired to a female that chose them and males paired with a female that did not make a choice. This made it possible to determine whether pair bond formation, or female preferences, had a role in a male’s stress hormone levels over time.

### **Behavioral Testing for Anxiety**

To examine behaviors indicative of anxiety in male subjects, two paradigms were used on day 14 following pairing with a female: the elevated plus maze (EPM) and the open field (OF) tests. Both the EPM and OF tests commonly have been used as a measure for anxiety in rodents, including prairie voles (Walf & Frye, 2007; Wang et al., 2009; Seibenhener & Wooten, 2015). EPM and OF tests use an anxiety-inducing agent: an unguarded, open area. In the wild, prairie voles commonly avoid such areas, as they would be more easily seen by predators. The EPM and OF tests measure anxiety by recording the number of entries into and time spent in the anxiety-inducing area. For the EPM test, the number of entries into and time spent in the open arms are commonly used to measure anxiety, as well as the latency period before each animal enters the open arms. In the open field test, the time spent in the “center,” open area of the apparatus is frequently used as a measure of anxiety (Carola et al., 2002). Measures indicative of locomotor activity or exploratory behavior (distance traveled, etc.) may be used as well. An increase in activity in the aversive areas indicate non-anxious behavior (Walf & Frye, 2007).

Animals were isolated for one hour before behavioral testing to obtain the final fecal collection for ELISA testing, as described. OF tests took place immediately before the EPM test and lasted 10 minutes. The OF apparatus consisted of a plastic chamber (56x56x20 (H) cm) with

the floor of each chamber subdivided by lines into 16 squares (14x14 cm). Before and after each test, chambers were cleaned with 70% ethanol solution. During testing, each animal was placed in the center of the chamber. The time that subjects spent in each of the squares, as well as the number of entries into each square (measured when the center point of the animal's body entered the square), were recorded using Noldus live video tracking software. Animals were recorded as being less anxious if they spent more time in the inner squares of the apparatus, while animals were considered more anxious if they spent more time in the outer squares of the apparatus. Animals that exhibited more exploratory behaviors (based on frequency of entries into each square, distance traveled, etc.) were thought to be less anxious than animals that exhibited less exploratory behaviors.

The EPM apparatus consisted of two open arms (35x6.5 cm) and two closed arms (35x6.5x15 (H) cm) that crossed in the center, and was elevated 45 cm off the floor. Each subject was placed in the center of the apparatus and allowed to explore for five minutes. The time spent in each arm, as well as entries into each arm and latency to enter the open arms (measured when the center point of the animal's body entered each arm) was recorded using Noldus live video tracking software. Each animal underwent two five-minute trials on the EPM apparatus, with approximately 20 minutes between each trial. If an animal jumped off the apparatus during testing, that trial was ended and a third trial was performed 20 minutes later. The apparatus was cleaned with 70% ethanol solution between each trial. Animals that spent more time in the open arms, a shorter latency to enter the open arms, and increased activity (measured by frequency of entries into the open arms) were considered less anxious than animals that spent more time in the closed arms.

Following the end of behavioral testing, males were terminated by being placed in a CO<sub>2</sub> chamber for five to 10 minutes. Following CO<sub>2</sub> exposure, males were decapitated. Trunk blood was collected from each male and stored for future experiments.



## **Females and Pregnancy**

Previous research has shown that for a robust, long-lasting pair bond to develop in prairie voles, the female needs to become pregnant within two to three days after being housed with a male (Curtis, 2010). For this reason, females were retained for 30 days, or until their first litter was born. The date of birth for the first litter was used to assess when females became pregnant, and this information was used to estimate whether a male had formed a long-lasting pair bond with his partner. Fecal corticosterone measurements across time, as well as the results of the OF and EPM tests, were compared between males that formed a successful, long-lasting pair bond, and those that did not.

## **Statistical Analysis**

### *Corticosterone Measurements*

Statistical analysis of all results was performed using Statistica or Excel software. During preliminary testing, measurements of fecal and plasma corticosterone were compared using a one-way ANOVA. Measurements for plasma corticosterone between groups exposed to different resident-intruder paradigms were also compared using a one-way ANOVA. Comparisons of measurements for fecal corticosterone over a 24-hour period after corticosterone injections were made using a repeated-measures ANOVA. Finally, measurements of fecal corticosterone over a 14-day period between bonded and non-bonded males, as well as between males paired with a female that chose them and males paired with a female that did not make a choice, were compared using two-factor repeated-measures ANOVAs.

### *Mate Choice Test*

Results from the mate choice tests were assessed in several ways. The numbers of animals that chose to spend time with the stressed male, the non-stressed male or alone were

compared using a chi-square test. The time females spent in physical contact with both males was compared using a Wilcoxin Signed Rank Test. The time females spent in each of the three cages was compared using a repeated measures ANOVA. Locomotor activity, measured by number of crossings into the different cages, was compared between groups of animals using an unpaired t-test.

#### *Aversion Test*

The expected number of females that chose to spend time with the male or in the empty cage were compared with the observed number using a chi-square test. The time females spent in each cage was compared using a repeated measures ANOVA. Time females spent in physical contact with the stressed vs. non-stressed male was compared using a Kruskal-Wallis test. Locomotor activity, measured by number of crossings into the different cages, was compared between groups using an unpaired t-test.

#### *Behavioral Tests*

Time spent in the inner and outer squares of the open field (OF) test was compared between males that had formed pair bonds and males that had not formed pair bonds using a repeated measures ANOVA. Distance traveled, a measure of locomotor activity in the OF test, was compared between groups using a one-way ANOVA. The frequency of entries into the inner and outer squares, another measure of locomotor activity in the OF test, was measured using a one-way ANOVA. For the elevated plus maze (EPM) test, time spent in the open arms vs. time spent in the closed arms was compared between the “bonded” and “non-bonded” groups using a repeated measures ANOVA. Locomotor activity, measured by the frequency of entries into the open and closed arms, as well as total distance traveled within the apparatus, was compared between groups using a one-way ANOVA. Latency to enter the open arms was also compared between “bonded” and “non-bonded” males using a one-way ANOVA.

### *Pairing Data*

Following the 30 days in which females were kept prior to the estimated birth of their first litter, animals were separated into “bonded” and “non-bonded” groups. Demographic data comparing the age and weight of females and male partners within these groups were compared using one-way ANOVAs and unpaired t-tests. Females that had been paired with a preferred “stressed” male, “non-stressed” male, or were randomly paired with one of the two males after not forming a preference in the mate choice test were noted. The number of females that gave birth within the time necessary for a long-lasting pair bond to be inferred was compared with the expected number using a chi-square test. Likewise, the number of females within the “stressed,” “non-stressed,” or “ambivalent” preference groups that gave birth within the allotted time were compared with expected numbers using a chi-square test.

Any pairwise comparisons conducted following a statistically significant result were performed using a Dunnett’s test or Tukey’s HSD test where appropriate. For all statistical analysis, a p-value of 0.05 was considered statistically significant.

## CHAPTER IV

### METHODS DEVELOPMENT

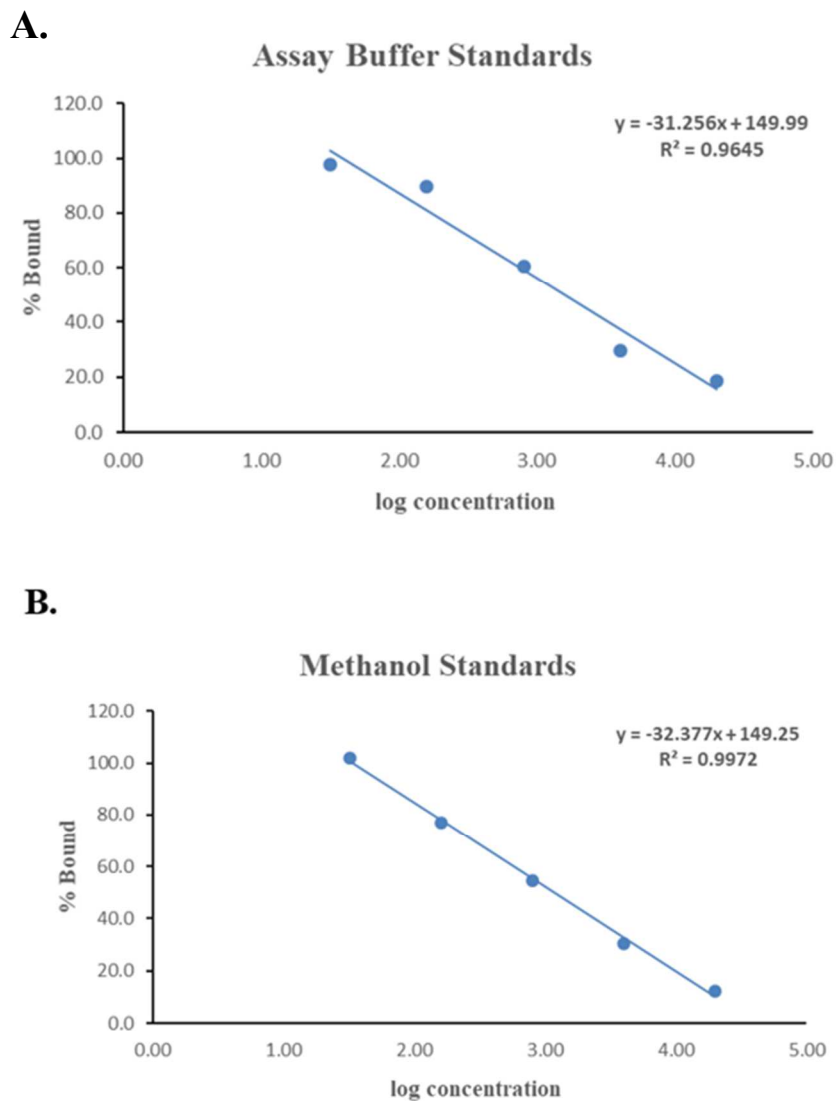
As stated, the goal of this project was to examine the interactions between stress and social bonding in prairie voles. The first experiment focused on the effect of acute stress in males on mate choice by females. To test this experiment, a stressor first had to be selected that would reliably elevate plasma corticosterone levels in male prairie voles. The second experiment involved studying the effects of pair bond formation on stress hormone levels over time. To accomplish this, it was necessary to measure corticosterone levels at multiple time points within the same animal. This chapter discusses the process of developing different approaches to carry out the above goals, and identifying the techniques ultimately used in this project.

## **Fecal Corticosterone Measurements**

Enzyme-Linked Immunosorbent Assay (ELISA) testing has previously been used to measure corticosterone in the plasma of rodents (Abelson et al., 2005; Zardooz et al., 2010; McNeal et al., 2017). Plasma collection in prairie voles usually requires the termination of the subject, only allowing for a single collection. However, this project required repeated measurements of corticosterone from a single subject across time. For this reason, it was necessary to develop a new method for measuring corticosterone in prairie voles. The following describes the use of ELISA kits to test for corticosterone in prairie vole feces in an effort to use a less invasive technique.

Fecal samples were prepared for ELISA testing using methanol extraction, following a protocol that had previously been used by our laboratory to measure estrogen in feces. Prior to testing fecal samples, a set of corticosterone standards (containing variations of diluted corticosterone reagent, as outlined in the Enzo ELISA kit protocol) in methanol were compared with corticosterone standards diluted in assay buffer. The standard curve was similar for both samples, and it did not appear that methanol had an effect on corticosterone measurements (Figure 2). Thus, methanol extractions of fecal samples were used to test for fecal corticosterone levels.

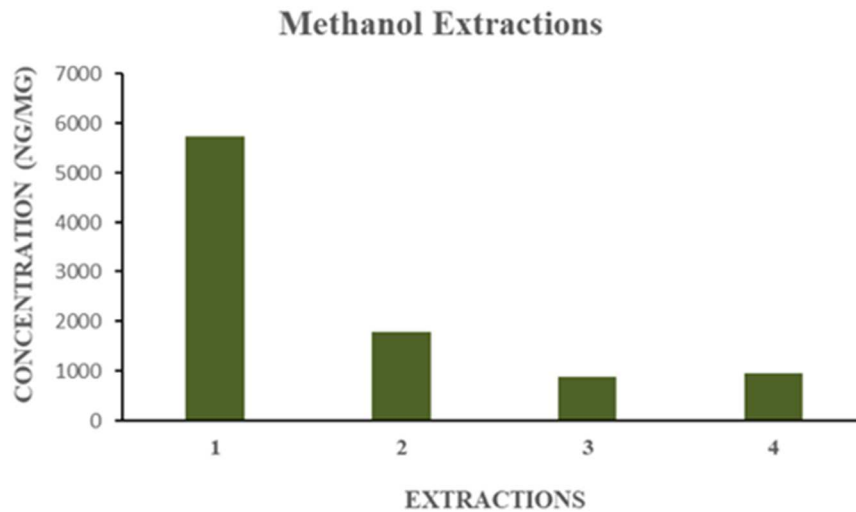
Fecal samples were collected from six male prairie voles and prepared using methanol extractions. Briefly, 0.1 grams of feces was placed in an Eppendorf tube and dried for one hour in a centrifuge vacuum. Following this procedure, the Eppendorf tubes were again weighed, and 500  $\mu$ l of 90% methanol solution was added. Following the addition of methanol, tubes were then placed on a mixer for one hour. At the end of this hour, the mixture in the tubes were



**Figure 2: Standard curves for corticosterone ELISA standards.** A) Corticosterone standards in assay buffer. B) Corticosterone standards in methanol solution.

sonicated, another 500  $\mu$ l of methanol was added, and the solution was vortexed. After vortexing, the tubes were centrifuged at 2500 rpm for 20 minutes. The supernatant was then extracted and placed in another Eppendorf tube. An additional one ml of methanol was added to the precipitant and centrifuged at 2500 rpm for another 20 minutes before being extracted. A total of four

extractions were originally tested to determine corticosterone content in this experiment.

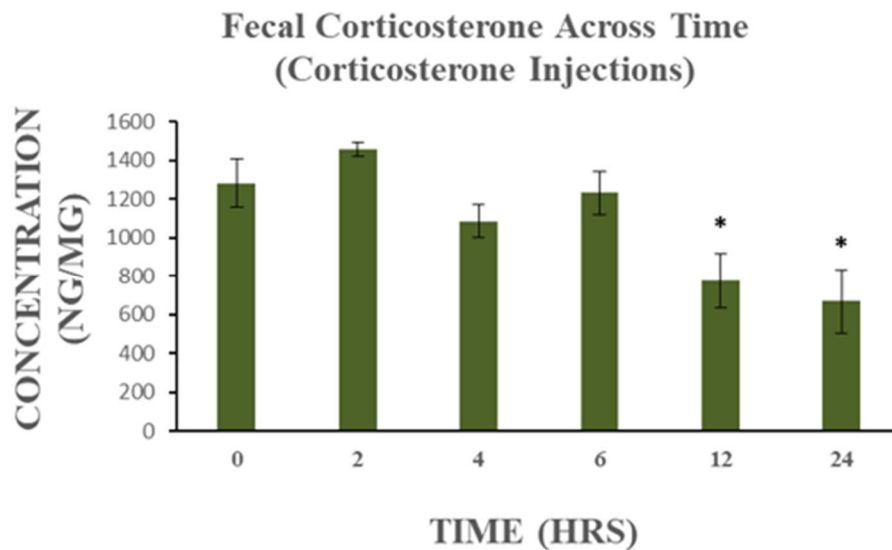


**Figure 3: Corticosterone extractions.** The majority of corticosterone was measured in the first and second extractions.

ELISA results showed that the majority (80%) of fecal corticosterone was in the first two methanol extractions (Figure 3). As such, the first two extractions of fecal samples were combined in subsequent tests. 30  $\mu$ l of this sample was dried in the speed vacuum to create a 1:100 dilution of the final sample. Following the drying process, 150  $\mu$ l of assay buffer and 150  $\mu$ l of 1:100 diluted steroid displacement reagent completed the sample. Following this procedure, samples could then undergo the ELISA procedure outlined by the Enzo corticosterone ELISA kit.

Next, the length of time required for an increase in plasma corticosterone to produce an increase in fecal corticosterone was tested. Six male voles were given intraperitoneal injections of 200  $\mu$ g of corticosterone. Fecal samples from subjects were collected immediately prior to injection, and then at two, four and six hours after injection. Fecal samples also were collected 12 and 24 hours following corticosterone injections. Fecal collection periods consisted of one hour during which subjects were isolated in an empty cage. All samples were tested for corticosterone

using the above method for measuring fecal corticosterone. Following a slight increase in fecal corticosterone two hours after injections, there was a continual decrease of fecal corticosterone throughout the 24-hour period following corticosterone injections (Figure 4). Measurements across the 24-hour time period were statistically significant ( $F_{5, 25} = 7.34$ ,  $p < 0.01$ ), and a Dunnett's test revealed a statistically significant decrease from baseline at hours 12 ( $p < 0.02$ ) and 24 ( $p < 0.01$ ).



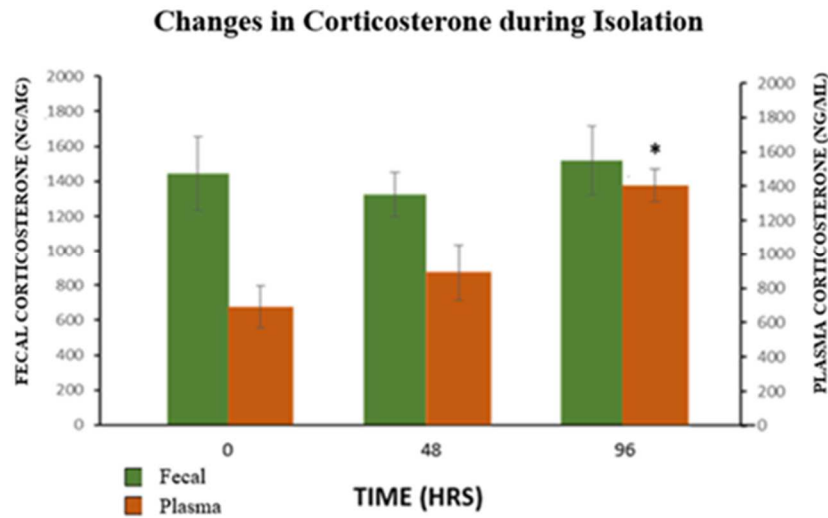
**Figure 4: Measurements of fecal corticosterone during a 24-hour period following corticosterone injections.** Fecal corticosterone was significantly lower than baseline measurements at 12 and 24 hours, as indicated by the asterisks ( $p < 0.05$ ).

### Acute Stressors

For females to have a choice between a stressed vole and a non-stressed control animal, an appropriate stressor needed to be used in this experiment. To find this stressor, several protocols were tested. Given that the measure for stress was increased plasma and/or fecal corticosterone, it was necessary to identify the stressor that caused the greatest increase in corticosterone. Isolation has been used to induce behavioral and neuroendocrine signs of anxiety



and depression in prairie voles (Grippe et al., 2007; Wang et al., 2009). For this reason, isolation was tested as a potential stressor to increase plasma and fecal corticosterone. Male voles were isolated in individual cages with bedding and were provided food and water *ad libitum*. Baseline samples from voles that had experienced no time in isolation were compared with those from animals that had been isolated for 48 hours and animals that had been isolated for 96 hours (Figure 5).



**Figure 5: Measurements of fecal and plasma corticosterone across time.** Measurements for fecal and plasma corticosterone were taken for animals at baseline, 48 hours of isolation, and 96 hours of isolation. 96 hours of isolation showed a significant increase in plasma corticosterone from baseline, as indicated by the asterisk ( $p < 0.05$ ).

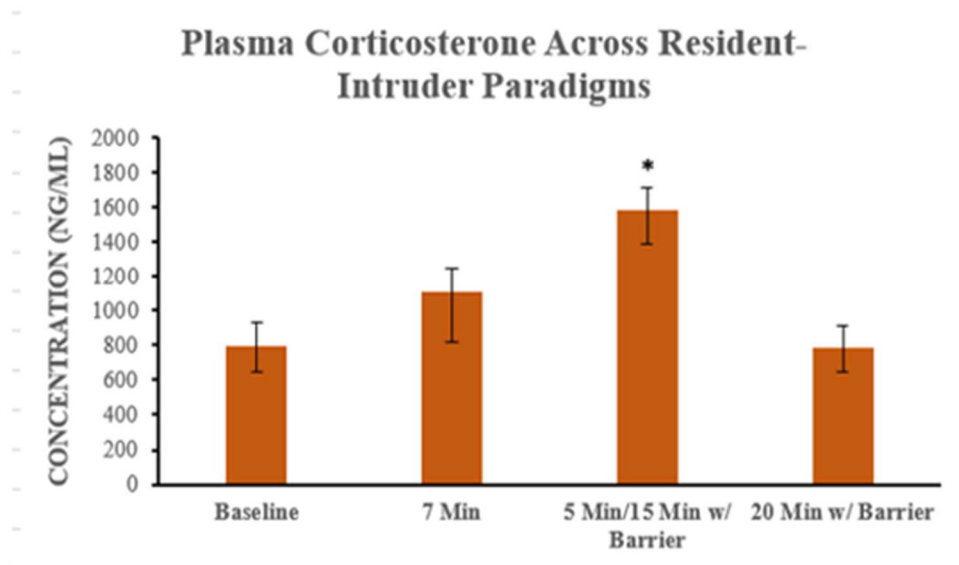
There were no significant differences in fecal corticosterone between the three groups of animals ( $F_{2,24} = 0.17$ ,  $p = 0.84$ ; Figure 5). There was, however, a steady increase in plasma corticosterone following increased time in isolation, and there was a significant difference in plasma corticosterone between the three groups of animals ( $F_{2,53} = 3.52$ ,  $p < 0.04$ ; Figure 5). Pairwise comparisons using a Dunnett's test revealed that 96 hours of isolation produced a significant increase in plasma corticosterone ( $p < 0.03$ ). While isolation appears to increase

plasma corticosterone, the effect was only observed following 96 hours of isolation. Thus, it was concluded that this would be more of a chronic stressor than an acute stressor. Since the aim of the project was to determine the effect of acute stress of males on mate choice by females, this would not be an ideal stressor for this project.

Resident-intruder encounters have also been used as a stressor in rodents. During a resident-intruder encounter, one male (the “resident”) is exposed to a novel, unrelated animal (the “intruder”) that is placed in the cage (Koolhaas et al., 2013). Along with different isolation periods, different resident-intruder encounters were tested to determine whether they could be used to increase fecal and plasma corticosterone. Initially, a 10 minute resident-intruder encounter was tested, where a male resident was exposed to an unrelated male in the same cage. Prairie voles that have mated show aggression toward unfamiliar conspecifics (Carter et al., 1995). Thus, mated males were chosen from our breeding colony to be the “intruders” in order to increase the likelihood of a stressful encounter. However, due to the level of aggression exhibited by the mated males, the initial encounter was ended at seven minutes, instead of ten, to avoid injury to the resident. Immediately following the test, residents were exposed to CO<sub>2</sub> gas and decapitated, their trunk blood collected to test for plasma corticosterone.

Due to the potential for injury to residents from the intruders, another protocol for a resident-intruder encounter needed to be developed. To address this need, two new methods were tested: first a protocol was tested where subjects underwent a resident-intruder encounter for five minutes, followed by 15 minutes of sharing a cage with the intruder, but separated by a wire mesh barrier. A second protocol was tested in which residents shared the same cage with an intruder, but were separated by a wire mesh barrier for a total of 20 minutes. Residents were exposed to CO<sub>2</sub> gas and decapitated, their trunk blood collected for plasma immediately following the different tests.

Comparisons between resident-intruder protocols revealed a statistically significant difference ( $F_{3, 22} = 3.76$ ,  $p < 0.03$ ; Figure 6). Pairwise comparisons using a Dunnett's test revealed that the only treatment that differed significantly from baseline was the five minute resident-intruder encounter followed by 15 minutes of sharing the same cage while separated by a wire mesh barrier ( $p < 0.02$ ). As it was the only short-term stressor that reliably produced a significant increase in plasma corticosterone across experiments, it was decided that the five minute resident-intruder encounter followed by 15 minutes of sharing the same cage while separated by a wire mesh barrier would be the acute stressor used in this project.



**Figure 6: Measurements of plasma corticosterone among different resident-intruder encounters.** Exposure to an intruder for five minutes, followed by sharing the same cage with an intruder while separated by a barrier for 15 minutes, caused a significant increase in plasma corticosterone from baseline, as indicated by the asterisk ( $p < 0.05$ ).

## Conclusion

The results of the experiments described in this chapter provided the information necessary to carry out this project. Repeated ELISA testing showed that fecal samples could

reliably be used to measure corticosterone in prairie voles. Thus, fecal samples were used to measure corticosterone across multiple time points. The experiments described above showed that the most reliable acute stress paradigm to elevate plasma corticosterone was the five minute resident-intruder test followed by 15 minutes of sharing the same cage, but separated by a wire mesh barrier. For this reason, this protocol was used as the acute stressor prior to the mate choice experiment for this project. The techniques developed in this chapter allowed for the opportunity to study the effect of acute stress in males on mate choice by females, and examine the effects of pair bond formation on levels of the stress hormone corticosterone across repeated time points.

## CHAPTER V

### FEMALE PRAIRIE VOLES PREFER MALES THAT HAVE NOT BEEN EXPOSED TO A STRESSOR

#### **Abstract**

In many species, chronic stress can influence mate choice by affecting the traits used to identify individual preferences. The prairie vole (*Microtus ochrogaster*) is a monogamous rodent known for its social behavior. The effects of stress on pair bonding in prairie voles are sexually dimorphic: exposure to a stressor or the stress hormone corticosterone reduces the likelihood of pair bond formation in females, but increases the likelihood in males. In this experiment, a series of mate choice tests were conducted to examine the effect of stress in males on mate choice by females. Modified “aversion tests” were conducted to determine whether female prairie voles are averse to stressed males. Females spent greater time in physical contact with males that had not been exposed to a stressor. Females also spent greater time in the cage containing a control male in the mate choice apparatus and preferred an empty cage to the cage containing the stressed male. There were no differences between the stressed and non-stressed groups in the aversion tests. The results of this experiment supported the hypothesis that stress in males can affect mate choice by females: females prefer males that have not been exposed to a stressor.

*Key words: prairie vole, stress, mate choice, aversion*

## **Introduction**

Many species rely on mate choice for reproduction. Mate preferences are based on a multitude of factors, including physical characteristics such as feather or coat color, genetic differences, or behavior (Williams, 2001; Kokko et al., 2003; Byers & Kroodsma, 2009; Sun et al., 2013). Environmental conditions may play a role, leading to selective pressures that can influence female preferences (Robinson et al., 2012). While there have been many studies on mate choice, more information is needed on factors affecting mate preferences in different species.

One such factor is stress. Chronic stress tends to have a negative effect on mate choice: in many species, individuals display preferences for potential mates that do not suffer from stress. Chronic stress can negatively influence an organism's health and immunity, making them more susceptible to disease (Guilliams & Edwards, 2010). This negative impact on overall health and vitality can also affect characteristics frequently used to determine sexual preferences, such as the quality of bird song or vocalizations (Husak & Moore, 2008). These negative influences could explain many species' preferences for individuals that have not undergone chronic stress.

Prairie voles are highly social rodents that form monogamous "pair bonds," mating for life with a single partner. Extensive research has been done to determine different factors affecting the formation of pair bonds in this species, and in fact, the effect of stress on pair bond formation in this species has been previously examined in some contexts. For example, studies have shown that males injected with corticosterone or exposed to an acute stressor were more likely to form lasting partner preferences with a mate than those who had not been stressed (Devries et al., 1996). A proposed explanation for this finding was that in the wild, males stressed from overcrowding in the natal nest would be more likely to disperse and find a mate. Females, on the other hand, may benefit from overcrowding-being able to mate with unfamiliar

conspecifics close by, while living in protection in the natal nest. However, the effects of stress on mate choice in this species have not been examined.

This study conducted mate choice tests to determine the effect of stress in males on mate choices by female prairie voles. While males can also have mate preferences, female preferences were tested to determine whether the positive influence of stress on social bonding observed in males could be due to female mate choice (Devries et al., 1996). Exposure to a chronic stressor could influence body condition and add compounding factors to a female's choice. For this reason, a model of acute stress exposure for the males was selected so that the only variable in the choice experiment was the presence or absence of acute stress (as opposed to differences in health or physical characteristics, as might be expected if a long-term stressor was used). The effects of stress on mate choice in other species led me to hypothesize that acute stress in males would influence mate choice by females. While stress in males tends to negatively influence mate choice in females of many species, the fact that stress enhances partner preference formation in male prairie voles led me to predict that females would prefer males exposed to an acute stressor (Devries et al., 1996; Husak & Moore, 2008).

## **Methods**

### *Stress Exposure*

Male prairie voles were separated into two groups: those that were to be exposed to an acute stressor (as measured by increases in plasma corticosterone in preliminary experiments), and those that were not stressed. All males were isolated for approximately one hour prior to the beginning of the experiment, around 1100 hours, for collection of the first fecal sample as required for the second experiment in this project. Those exposed to an acute stressor underwent the paradigm described in the general methods section, where the subject was exposed to a mated male for five minutes, and then separated from the mated male by a wire mesh barrier for 15

minutes. Following stress exposure, all males, both stressed and non-stressed, were tethered in individual cages during the female mate choice test.

#### *Mate Choice Testing*

The mate choice procedure was as described in the general methods section. Preferences of a total of 23 female voles were tested during this experiment. Video recordings were viewed immediately after the end of the test, and females were paired with the male they preferred. A female was said to have a preference for a specific male if she spent 10 or more minutes in physical contact with him. If a female did not show a preference for either male, then she was randomly paired with one or the other of the males.

#### *Aversion Testing*

Based on the results of the mate choice test, it was unclear whether females simply did not prefer stressed males, or were averse to them. For this reason, a series of tests were conducted to determine whether females displayed an aversion to males exposed to acute stress. Animals were subjected to the “aversion test” described in the methods section. Similar to the mate choice test, the aversion test lasted for a total of two hours during which female voles were allowed to explore the apparatus. The test was video recorded for later review, and cage time and number of cage crossings were also measured.

#### *Statistical Analysis*

The number of females that chose to spend more time with the stressed or non-stressed control male were assessed using a chi-squared test. The amounts of time females spent with the stressed male vs. with the non-stressed male were compared with a Wilcoxin Signed Rank Test. The amounts of time females spent in the different cages were compared using a repeated measures ANOVA. Locomotor activity (cage crossings) was compared using an unpaired t-test.



Time spent with the stressed or non-stressed control males during the “aversion test” was assessed using a Kruskal-Wallis test. Further statistical analysis of the aversion test consisted of group comparisons between the time females spent in each cage using a repeated measures ANOVA, and comparisons between groups for locomotor activity using unpaired t-tests. Age and weight differences between animals that had pups within 23-25 days and those that did not were compared using one-way ANOVAs and unpaired t-tests. Lastly, the number of animals that had pups within the allotted time within each group were assessed using a chi-squared test. Comparisons were considered statistically significant if  $p < 0.05$ .

## **Results**

### *Mate Choice Test*

Of the 23 females that underwent the mate choice procedure, only two females chose to spend 10 or more minutes in contact with the stressed male, while 11 chose to spend 10 or more minutes with the non-stressed control male. The remaining 10 females did not spend 10 or more minutes with either male. These latter 10 females were designated “ambivalent” females that did not make a choice. A chi-squared test was used to assess the number of females that preferred one or the other male, and a significantly greater number of females chose the non-stressed males over the stressed males ( $p < 0.02$ ). Females also spent significantly greater time in physical contact with the non-stressed control male than with the stressed male ( $Z = 2.09$ ,  $p < 0.04$ ; Figure 7A).

The amount of time subjects spent in each cage varied significantly as well ( $F_{2, 40} = 5.22$ ,  $p < 0.01$ ; Figure 7B). Pairwise comparisons revealed that, of the animals that made a choice, females spent a significantly greater amount of time in the cage of the “non-stressed” control male than in the other two cages ( $p < 0.01$ ). Of the “ambivalent” animals (those that did not make a choice), females spent significantly greater time in the empty, center cage than in the cage of

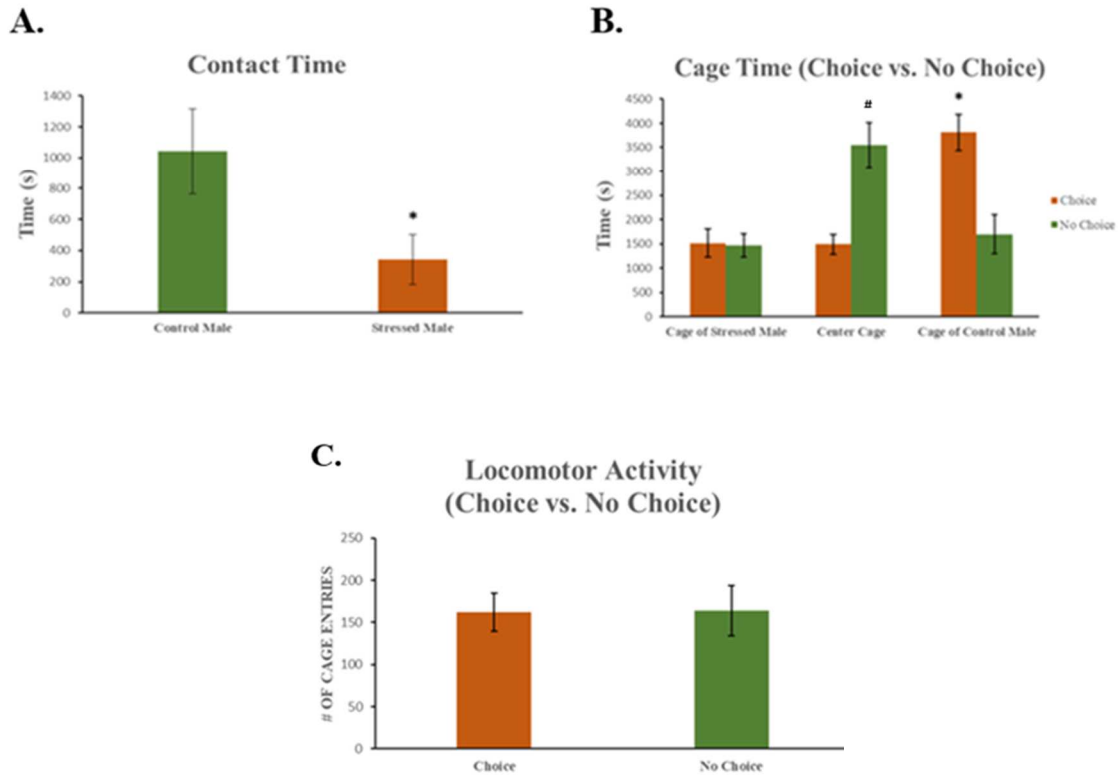
either male ( $p < 0.1$ ). Locomotor activity did not differ between females that chose to spend time with either male, and females that did not make a choice (Figure 7C). There were no significant differences in the total number of cage crossings between these two groups ( $t(20) = 0.04$ ,  $p = 0.96$ ).

#### *Aversion Test*

Comparisons between animals that had the choice of spending time with a stressed male or alone, and animals that had the option of being with a non-stressed control male or alone yielded no significant differences. There were no differences for the amount of time females spent in physical contact with either male ( $H(1) = 0.03$ ,  $p = 0.87$ ; Figure 8A). Nor were there any differences in cage time between the two groups: females did not differ in the time spent in either the empty cage or the cage with a male present ( $F_{1, 15} = 0.50$ ,  $p = 0.50$ ; Figure 8B). Finally, there were no differences between groups for locomotor activity ( $t(15) = 0.40$ ,  $p = 0.70$ ; Figure 8C).

#### *Gestation*

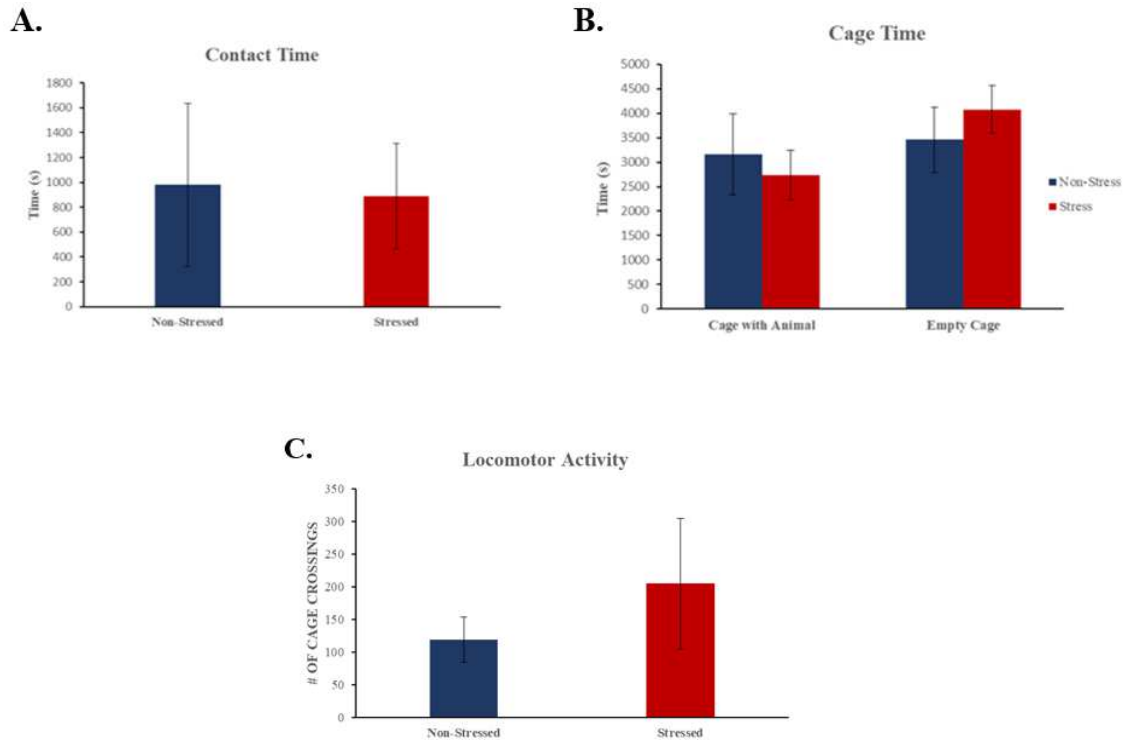
Following the mate choice procedure, animals were paired and housed together for 14 days. Females that “chose” a male during the mate choice procedure were housed with their preferred male and kept for at least three weeks to determine latency to parturition. Of the females that did not make a choice, half were paired with the stressed male and half were paired with the non-stressed male. Of the 21 females that remained paired with a male throughout the 14-day period following the mate choice procedure (two were terminated after sustaining injuries), 10 gave birth within 23-25 days following pairing. Six of the nine females that chose the non-stressed male had pups within 23-25 days. Of the two animals that chose the stressed male, neither had pups within the allotted timeframe. Of the 10 animals that did not make



**Figure 7: Results of the mate choice test.** A) Females spent more time in contact with the non-stressed male than the stressed male B) Females that made a choice between males spent more time in the cage of the non-stressed control male, while females that did not make a choice spent more time in the center cage. C) There were no differences in the number of cage crossings between females that chose a male and females that did not make a choice. Significance is represented by asterisks and the pound sign ( $p < 0.05$ ).

a choice, four had pups 23-25 days following pairing: two of these animals had been paired with a “stressed” male, and two with a “non-stressed” male (Table 1).

Differences between the observed numbers of animals in each group that had pups were assessed based on records from previous research (Curtis, 2010). A chi-squared analysis revealed that differences between groups were not statistically significant ( $p = 0.24$ ). Interestingly, only 11 females gave birth in this experiment, with less than half of females having pups during the allotted time period. A chi-squared analysis showed that these results were significantly different than expected ( $p < 0.02$ ).



**Figure 8: Aversion testing.** A) Time spent in physical contact with the stressed vs. non-stressed male. B) Time spent in each cage for females exposed to a stressed male, and females exposed to a non-stressed male. C) Locomotor activity. There were no differences for any factor between females that had the option of spending time with a stressed male or alone, or a non-stressed male or alone.

*Further Demographic Comparisons*

There were no demographic differences between animals that had pups within the allotted time period and animals that did not. Neither were there demographic differences in males or females between groups that chose the stressed animal, the non-stressed control animal, or did not make a choice. Females had an average age of 95 days at the beginning of the experiment, and there were no significant differences for female age between groups ( $F_{1,16} = 0.44, p = 0.51$ ) or between groups that had pups and those that did not ( $F_{1,16} = 0.73, p = 0.41$ ). Likewise, males had an average age of 88 days at the beginning of the experiment, and there were no significant

differences in age between males that were “chosen” by females and males that were not ( $t(43) = 0.33, p = 0.75$ ). There were no significant differences in male age between groups ( $F_{1, 11} = 1.01, p = 0.78$ ), nor between males that sired pups within the allotted time frame and males that had not ( $F_{1, 11} = 0.54, p = 0.84$ ).

Births per Group		
	Number of Females	Females that Had Pups in 23-25 Days
<b>Total</b>	<b>21</b>	<b>10</b>
<b>Chose "Stressed" Male</b>	<b>2</b>	<b>0</b>
<b>Chose "Non-Stressed" Male</b>	<b>9</b>	<b>6</b>
<b>No Choice (Total)</b>	<b>10</b>	<b>4</b>
<b>No Choice, Paired with "Stressed" Male</b>	<b>5</b>	<b>2</b>
<b>No Choice, Paired with "Non-Stressed" Male</b>	<b>5</b>	<b>2</b>

**Table 1: Births per group.** Number of females that gave birth in each group of pairings based on females’ choice. Note: females that did not make a choice were randomly paired with the stressed male they were exposed to during the mate choice procedure, or the non-stressed control male.

There also were no differences in weight between animals. The average weight for males that were paired was 38.0 g. There was no difference in weight between males that sired pups in the allotted time and males that had not ( $t(18) = 0.01, p = 1.00$ ), neither was there a difference in the weight between stressed males, non-stressed males and males that had been paired with ambivalent females ( $F_{1, 17} = 1.75, p = 0.20$ ). The average weight for females was 43.4 g. There was no difference in weight between females that had pups in the allotted time and females that did not ( $t(15) = 0.44, p = 0.66$ ), and neither were there any differences between the weight of females that chose the stressed male, the non-stressed male, or did not make a choice ( $F_{1, 14} = 2.06, p = 0.16$ ).

## **Discussion**

The purpose of this project was to examine the influence of acute stress in males on mate choice by females. During the mate choice procedure, the majority of females (87%) chose to spend time with the non-stressed male rather than the stressed male, or did not make a choice between the males. Likewise, females spent less time in the cages of the stressed males than they did in the cages of the non-stressed males or in the empty cages. Taken together, these results suggest that male exposure to an acute stressor can affect mate choice by females in this species, and that females seem to prefer males that have not been exposed to the acute stressor used in this study.

These results raised the question: do females simply prefer males that have not been exposed to a stressor, or do they actually have an aversion against stressed males? The results of the aversion test, in which a female was allowed the choice of spending time with a single animal (stressed or not) or in an empty cage, seem to suggest the former. There were no significant differences between the number of animals that chose to spend time with the stressed male vs. an empty cage, and the number of animals that chose to spend time with the non-stressed control male vs. an empty cage. It appears that females may simply prefer a male that hasn't been stressed, as opposed to having an aversion to stressed males. This would also argue against the possibility that exposure to stress elicited unusual aggression by the male that could "chase" the female away.

The original hypothesis was that stress in males would have an effect on mate choice by females. These experiments support this hypothesis: the majority of females prefer a male that had not been exposed to a stressor. The original prediction, however, was incorrect. Previous research has indicated that male prairie voles injected with corticosterone or exposed to a stressor are more likely to form a lasting "partner preference" or pair-bond (Devries et. al, 1996). This

observation led to the prediction that females would prefer a male that had been exposed to an acute stressor over a male that had not been stressed. The opposite occurred, however: of females that chose to spend time with a male, most preferred males that had not been stressed. With the results not supporting the earlier prediction, it was concluded that acute stress in males must affect some other characteristic important for pair bond formation, such as behavior. Devries and colleagues (1996) suggested that stress from overcrowding in the nest may lead prairie voles to disperse and find mates. This proposal requires further examination in the future.

While these results may have contradicted the original prediction based on earlier research with voles, they align with much of the research on stress and mate choice across the animal kingdom. Female animals in general prefer males that are not stressed. Stress, particularly chronic stress, can have a negative impact on mate choice: females generally prefer males with good body condition, and increased levels of circulating glucocorticoids, especially over a prolonged period of time, can negatively influence body condition. Chronic stress can negatively affect traits often used for sexual selection in different species; elevated circulating glucocorticoid levels, for instance, have been found to negatively influence song quality in birds (Husak & Moore, 2008). While this experiment focused on the effects of acute stress, exposure to a stressor still appeared to have a negative impact on female mate choice. The reasons behind this are unknown, although behavioral signals or pheromones could have played a role in this effect. Nevertheless, stress in males appeared to have a negative impact on mate choice by females in this study, following a trend frequently seen in the mate choice literature (Husak & Moore, 2008; Bonier et al., 2009).

An unexpected result of this experiment was the number of females that did not make a choice. Of the 23 females originally tested, 10 chose not to spend 10 or more minutes with either male during the mate choice protocol. As previously discussed, prairie voles are a highly social species, and the majority of animals in earlier experiments have preferred to spend time with a

conspecific rather than be alone (Carter et al., 1995). In fact, the results of the aversion test are consistent with such observations. The results of this experiment, then, are somewhat unusual compared to other research.

A number of studies utilizing social preference tests in prairie voles first artificially induced estrus in females to make them sexually receptive to males. This can be done by injecting females with estradiol benzoate, or exposing them to soiled bedding from a male (Carter et al., 1987; Wang et al., 1999; Lim & Young, 2004). In the present experiment, females were intact and not estrogen-primed, thus mimicking natural conditions. Initially, it was believed that this fact could account for the differences seen in this experiment. However, there have also been studies that used intact females that were not in estrus, and the results from such partner preference tests still differed from ours (Devries et al., 1996; Curtis, 2010).

Many of the partner preference tests in the previous literature have examined effects of familiarity amongst pairs. In particular, in studies focusing on the neuroendocrine effects of pair bonding, animals were housed as pairs prior to the partner preference test, sometimes for an hour of cohabitation immediately before the test, and other times longer (Devries et al., 1996; Cho et al., 1999; Aragona et al., 2003). The effects of familiarity toward one of the tethered animals in these tests could account for some of the differences in results from previous social preference experiments and our own. However, not all studies examining partner preferences and mate choice in prairie voles have required cohabitation before the tests, so this does not fully explain the reason for our differences.

The mate choice protocol used in this experiment is new, having been developed for this study. The more typical partner preference test takes place over a period of three hours, while the mate choice paradigm we used is only two hours long. Initially, it was thought that this one-hour difference might explain our results in regard to the animals that did not make a choice: perhaps



females did not have the time necessary to make a choice and settle down with a mate. However, previous data from our lab shows that, if a female is going to make a choice, it will be made within the first two hours of the test. The difference of one hour does not appear to have an effect on the results of the test. The appearance of these “ambivalent” females that do not make a choice may require further study in the future.

Another uncommon result was the females’ birth rate following the mate choice experiment. Previous work from our lab has suggested that over 80% of pairs would be expected to have their first litter within 21-27 days of being paired, illustrated by one particular experiment in which 21 out of 25 pairs gave birth within this time frame (Curtis, 2010). However, of the 21 females kept until their first litter, only 10 had pups within the allotted time. Male stress and the ability to choose a mate might have had an effect. Of the nine females that chose the non-stressed control male, six had pups within 23-25 days of pairing, while neither of the two females that chose the stressed male had pups. Of the 11 females that did not make a choice and were randomly paired, four had pups during the allotted time period: two that had been paired with the stressed male, and two with the non-stressed control male. Unfortunately, there were not enough females in each group (particularly in the group that chose the stressed animal) to come to any definitive conclusions. Nevertheless, the effects of mate choice on pair-bond formation and fertility in this species merit further examination.

One caveat that should be mentioned is that, for several weeks, the timer controlling the light/dark cycle for the room housing experimental pairs was off by several hours, and animals were exposed to more light than usual. This could have affected pair bond formation and birth in the animals: however, animals that experienced the normal light: dark cycle displayed similar outcomes. For this reason, it seems unlikely that the change in photoperiod was the main reason for the differences in birth number. The effects of choice, stress or a number of other factors most likely contributed as well.

The results of this experiment seem to suggest that acute stress in males can have an effect on mate choice by females and potentially pair bond formation in prairie voles. Stress in males may negatively affect mate choice by females, following a trend seen in the mate choice literature across many different species. Allowing the female a choice in mates may also affect pair bond formation, as may initial stress in males. Further study is needed to investigate the effects of choice and stress on fertility and pair-bond formation in female prairie voles.

## CHAPTER VI

### PAIR BOND FORMATION INFLUENCES STRESS HORMONE LEVELS OVER TIME BUT DOES NOT AFFECT MOST STRESS BEHAVIORS

#### **Abstract:**

A major factor affecting health is stress: individuals suffering from chronic stress are more likely to develop a wide variety of diseases and disorders. Positive social relationships can influence stress levels, possibly acting as a “buffer” against the negative effects of stress. Prairie voles (*Microtus ochrogaster*) have previously been used to study stress and social bonds. In this experiment, the effects of pair bonding on the stress hormone corticosterone were examined in male voles. Two behavioral tests were conducted to determine the effects of pair bonding on stress responses in male prairie voles. Bonded males had lower corticosterone levels over time than non-bonded males. Results of the elevated plus maze test were mixed: there were no differences between bonded and non-bonded males for time spent in the different arms of the apparatus, but non-bonded males frequented the arms more often. Results of the open field test also varied: there were no differences between groups for time spent in the different areas of the apparatus, but bonded males traveled a greater total distance than the non-bonded males. Results supported the hypothesis that pair bond formation would influence stress hormone levels across time, but did not support the prediction that bonding would influence stress behaviors.

*Key words: Stress, pair bond, corticosterone, prairie vole*

## **Introduction**

Social relationships, such as marriage, can positively influence health. Studies have shown that individuals in happy marriages are generally healthier and live longer than their unmarried counterparts. There are two possible explanations for this phenomenon: selection and protection. “Selection” refers to the idea that individuals with good health are already more likely to marry. “Protection” refers to the explanation that marriage may add a protective benefit by affecting the social, psychological and physical environment around a person, positively affecting their health and health behaviors (Schone & Weinick, 1998).

Evidence also suggests that relationships can have physiological effects on health. Supportive social networks lead to benefits for the immune, endocrine and cardiovascular systems. Positive social support in childhood can promote healthy development of systems, such as the immune system, autonomic nervous system, and HPA axis. Individuals with social support in adulthood, particularly those who are married, also benefit from a lower risk of cardiovascular disease and reduced cardiovascular reactivity to different stressors (Taylor et al., 1997; Umberson & Montez, 2010).

Stress is a major factor that can influence health. Common effects of stress include negative emotions, increased physiological reactivity, and behavioral changes. The social buffering hypothesis suggests that one of the ways relationships can positively affect health is by providing a buffer against the negative effects of stress (Cohen & Wills, 1985). A healthy marriage, as well as other positive social relationships, may serve to decrease reactivity to certain stressors and serve as a buffer against many of the negative consequences of chronic stress.

Prairie voles are social rodents that form monogamous pair bonds. As such, they are an ideal animal model for studying the interaction between stress and social bonding. There have been multiple studies involving stress and anxiety in this species. Devries et. al (1996) found that

the effect of acute stress on pair-bond formation was sexually dimorphic: females exposed to a stressor or injected with corticosterone were less likely to form a long-lasting “partner preference,” while males exposed to the same protocol were more likely to do so.

Other studies have shown that social bonds, or a lack of them, can affect neuroendocrine and behavioral measures of anxiety. Grippo et al. (2007) showed that, following four weeks of social isolation, male and female prairie voles showed signs of anhedonia and had elevated circulating levels of oxytocin, adrenocorticotrophic hormone (ACTH) and corticosterone. In another study, male prairie voles exposed to six weeks of social isolation spent more time in the closed arms of an elevated plus maze (EPM) test and moved more frequently from the central to peripheral squares in an open field (OF) test, both of which are behavioral indicators of anxiety (Wang et. al, 2009). These changes suggest that social bonds can have an effect on neuroendocrine function and anxiety in this species.

While the above studies have focused on stress and social bonding, the effect of pair bond formation on stress has not been thoroughly examined in prairie voles. The following experiment attempts to address this gap by examining the effects of pair bonding on the stress hormone corticosterone (a common measure for stress in this species) across time, as well as on behaviors that are often used as measures for anxiety in rodents. In this experiment, fecal samples were collected from male voles to examine changes in corticosterone during a two-week period after being paired with a female. Following this two-week period, males were subjected to an open field (OF) test and an elevated plus maze (EPM) test to examine behavioral signs of anxiety. In this experiment, it was hypothesized that the formation of a monogamous pair bond would affect a male’s corticosterone levels over time, as well on behavioral signs of anxiety in the OF and EPM tests. The prediction was that there would be a steady decrease in fecal corticosterone over time in males that had formed a successful pair bond, and that bonded males would show fewer

signs of anxiety-like behavior during the OF and EPM tests when compared to males that had not formed a pair bond.

## **Methods**

### *Pairing and Fecal Collection*

Immediately following the mate choice test described in the previous chapter, the video recording of the test was watched to determine whether female voles had made a choice. Females were then paired with the male they preferred. If they did not make a choice between males, then they were paired randomly with either the stressed male or the non-stressed control male. After being paired, animals were housed together for 14 days. Fecal samples were taken from males around 11:00 a.m. on days 0, 1, 2, 3, 5, 7, 10 and 14 (pre-pairing fecal samples were collected prior to the choice test). Fecal samples were analyzed for corticosterone content via ELISA testing as described in the general methods section. Measurements of fecal corticosterone across groups were normalized by subtracting baseline (day 0) measures of corticosterone from the measures for other days samples were taken. After normalization, corticosterone measures over time were compared between bonded and non-bonded males, as well as males paired with a female that chose them and males paired with a female that did not make a choice.

### *Behavioral Testing*

On day 14 following pairing, males were subjected to behavioral testing. After fecal collection, males underwent a ten-minute open field (OF) test. Males were placed in the center of the open field, and their movements were tracked and recorded during the ten-minute period. Immediately following the open field (OF) test, males were subjected to two separated trials of elevated plus maze (EPM) tests. Each trial was five minutes long and the animals' movements were tracked and recorded during this period for later review. A number of males jumped off the

elevated plus maze during one or both trials. These males were subjected to a third five-minute trial as well.

### *Groups*

Following behavioral testing, the males were terminated. Females were retained until the birth of their first litter or 30 days after being paired. As previous research has indicated that females need to achieve pregnancy within two to three days of pairing for a robust, long-lasting pair bond to develop (Curtis 2010), the date of parturition was used to divide males into two groups: those that had formed a successful pair bond, and those that had not. Comparisons between the two groups were made to determine the effects of successful pair bond formation on corticosterone levels across time as well as behavioral signs of anxiety.

### *Statistical Analysis*

Measurements of fecal corticosterone across time between bonded and non-bonded males, as well as males that were paired with a female that “chose” them vs. an ambivalent female, were compared using two-factor repeated measures ANOVAs. Time spent in the different areas of the open field (OF) test between groups was compared using a repeated measures ANOVA. Distance traveled during the OF test was compared with an unpaired t-test. Time spent in the different arms of the elevated plus maze (EPM) test between groups was compared using a repeated measures ANOVA. Locomotor activity was compared using an unpaired t-test. Results were considered statistically significant if  $p < 0.05$ .

### **Results**

Only 21 of the 23 initial pairs were tested throughout the experimental period: two pairs were terminated prior to day 14 due to injuries sustained by the male. Of the 21 females that were paired, ten had pups by day 26 following pairing, indicating that these pairs had formed a

successful pair bond. Of the remaining 11 females, only one proceeded to have pups, on day 30 following pairing. These 11 pairs were placed into the “non-bonded” group and were compared with subjects that were designated as being pair-bonded when analyzing the results of the experiments described above.

### *Corticosterone Measurements*

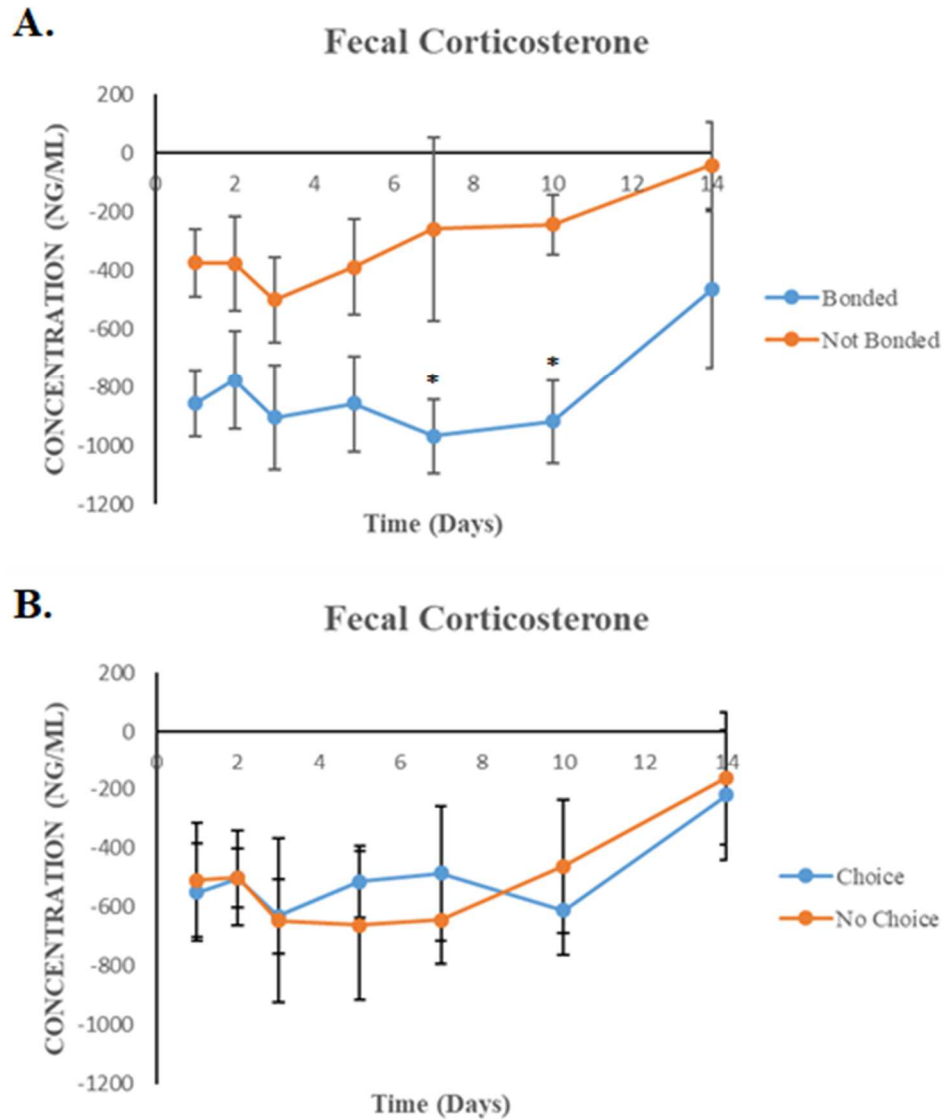
Comparisons of fecal corticosterone levels between bonded and non-bonded males over a 14-day period revealed bonded males had significantly lower corticosterone levels over time than non-bonded males ( $F_{1, 18} = 7.50$ ,  $p < 0.02$ ; Figure 9A). Pairwise comparisons utilizing Tukey’s HSD test revealed that bonded males had significantly lower corticosterone than non-bonded males on days 7 ( $p < 0.03$ ) and 10 ( $p < 0.05$ ). There was also a significant effect of time on corticosterone levels, with fecal corticosterone for both groups gradually increasing over the 14-day period ( $F_{6, 108} = 2.74$ ,  $p < 0.02$ ). Fecal corticosterone levels were also compared between males that had been paired with a female that chose them, and males that had been paired with a female that did not make a choice. There were no differences in fecal corticosterone over the 14-day period between these two groups ( $F_{1, 19} = 0.003$ ,  $p = 0.96$ ; Figure 9B), nor were there any effects of time on corticosterone levels over this period ( $F_{6, 114} = 2.13$ ,  $p = 0.06$ ), although there was a trend of fecal corticosterone levels increasing over time for both groups.

### *Open Field Test*

The results of the open field (OF) tests revealed no significant differences for time spent in the inner and outer squares of the apparatus between bonded and non-bonded groups ( $F_{1, 19} = 3.00$ ,  $p = 0.13$ ). There was a significant effect of time, however: both groups of males spent significantly more time in the outer squares of the apparatus than in the inner squares ( $F_{15, 285} = 10.0$ ,  $p < 0.01$ ; Figure 10A). There was a significant difference in total distance traveled between



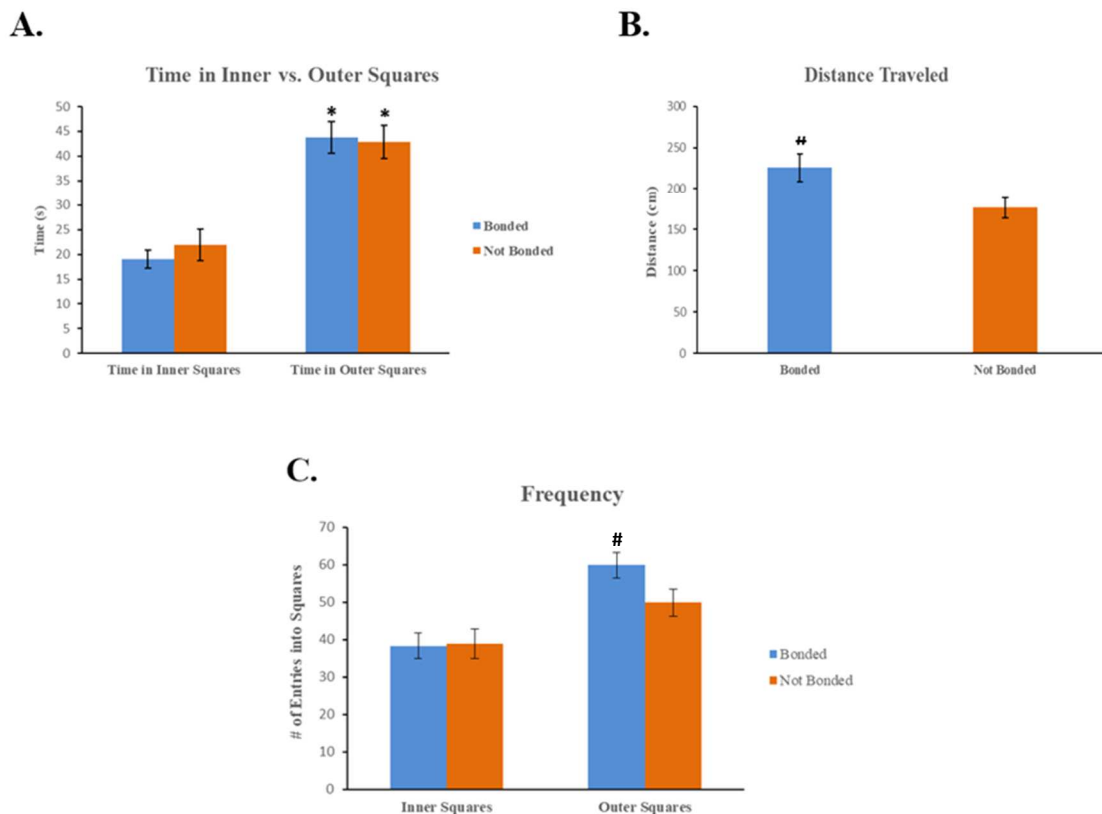
groups, with bonded males having traveled a greater distance than non-bonded males ( $t(296) = 2.30, p < 0.02$ , Figure 10B). There was also a significant difference in frequency, or the



**Figure 9: Fecal corticosterone levels over time.** A) Corticosterone levels between bonded and non-bonded males over time. B) Corticosterone levels between males paired with a female that chose them and males paired with a female that did not make a choice. Significance is indicated by asterisks ( $p < 0.05$ ).

number of entries into each square, between bonded and non-bonded males ( $F_{3, 332} = 7.33$ ,  $p < 0.01$ ).

Both groups entered the outer squares more frequently than the inner squares, and pairwise comparisons using Tukey's HSD test revealed that the frequency of entries for the bonded males into the outer squares differed significantly from frequency for the inner squares ( $p < 0.01$ ), as well as from the frequency of entries into the outer squares for the non-bonded males ( $p < 0.01$ ).



**Figure 10: Results of the open field test.** A) Time spent in the inner and outer squares for bonded and non-bonded males. B) Total distance traveled for bonded and non-bonded males. C) Number of entries into the inner and outer squares during the open field test for bonded and non-bonded males. Significance is indicated by asterisks and the pound symbol ( $p < 0.05$ ).

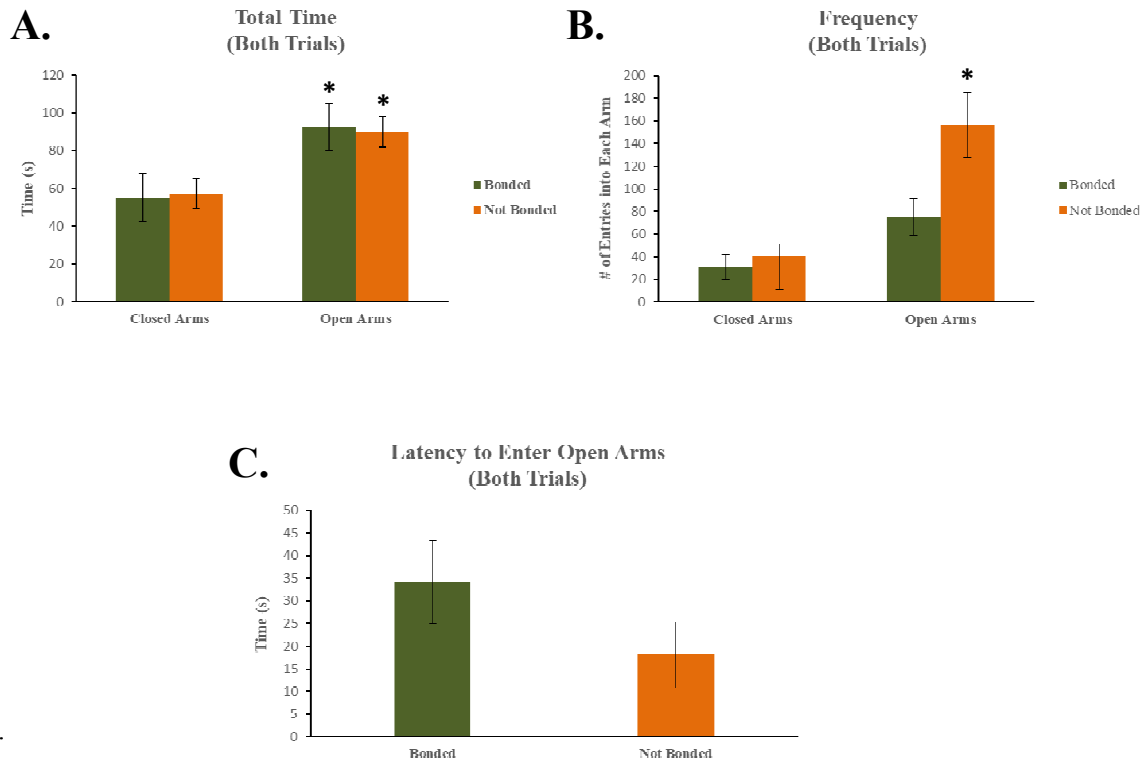
### *Elevated Plus Maze*

The results for trial one of the elevated plus maze (EPM) showed that, of the animals that completed the trial without jumping off, there were no significant differences between groups for time spent in either arm ( $F_{1, 14} = 0.08$ ,  $p = 0.78$ ). Males from both groups spent more time in the open arms than in the closed arms, although the time difference was not considered significant. The results for trial two of the elevated plus maze (EPM) were similar to those seen in trial one. There were no differences between bonded and non-bonded males for time spent in either arm ( $F_{1, 13} = 0.01$ ,  $p = 0.92$ ). When the total data from both trials was combined, there were again no significant differences between groups of animals for time spent in either arm ( $F_{1, 29} = 0.001$ ,  $p = 0.97$ ). However, there was a difference for time spent in each arm: overall, animals from both groups spent significantly more time in the open arms of the apparatus than in the closed arms ( $F_{1, 29} = 8.16$ ,  $p < 0.01$ ; Figure 11A).

The frequency of entries into the closed and open arms of the EPM apparatus were also compared between bonded and non-bonded males for both trials. Combined data from both trials revealed a significant difference between groups for frequency of entries into the different arms ( $F_{3, 60} = 9.34$ ,  $p < 0.01$ ; Figure 11B). Pairwise comparisons using Tukey's HSD test revealed that non-bonded males entered the open arms with greater frequency than the closed arms ( $p < 0.01$ ), as well as with greater frequency than the bonded males entered the closed ( $p < 0.01$ ) or open ( $p < 0.02$ ) arms. Lastly, the latency to enter the closed and open arms was compared between bonded and non-bonded males. There were no differences between bonded and non-bonded males for latency to enter either the closed or open arms of the apparatus ( $F_{3, 28} = 0.83$ ,  $p = 0.49$ ; Figure 11C).

In the first trial of the EPM test, five of the 21 subjects jumped off prior to the end of the five-minute period. Two of the five males were in the bonded group, while three were in the non-

bonded group. In the second trial, six of the 21 total subjects jumped off before the end of the five-minute period. Two of these animals had also jumped off of the apparatus prior to the end of the first trial. Of the six animals that jumped off before the end of the five-minute period during the second trial, half were males that had been bonded, and half were not. These animals were not included when analyzing the results of trials one and two of the EPM test.



**Figure 11: Results of the elevated plus maze (EPM) test.** A) Overall time in the closed arms vs. open arms for bonded and non-bonded males. B) Overall frequency of entries into the closed and open arms for bonded and non-bonded males. C) Overall latency to enter the open arms of the EPM apparatus for bonded and non-bonded males. Significant results are indicated by asterisks ( $p < 0.05$ ).

## Discussion

The purpose of this experiment was to determine the effects of pair bond formation on corticosterone levels across time, as well as behavioral measures of anxiety in male prairie voles. Initial ELISA results for fecal samples indicated that pair bond formation had a significant effect on corticosterone levels over time. There were significant differences between bonded and non-bonded males over a 14-day period: fecal corticosterone of bonded males was lower over time than the corticosterone of non-bonded males, showing significant differences on days 7 and 10. The combined results of this experiment support the original hypothesis: pair bond formation appears to influence stress hormone levels over time.

Prairie voles display an ultradian activity cycle (Lewis & Curtis, 2016), which could lead to variations in corticosterone levels depending on the time of day. However, fecal samples were collected at a similar time each day, so any differences in corticosterone would not be due to the voles' ultradian rhythm. Previous experimentation with fecal extractions showed that ELISA testing could be used as a suitable method to measure corticosterone levels. Thus, any observed changes in corticosterone were not due to time of day or the methods used for measuring corticosterone, but to other factors-notably, the influence of pair bonding.

Results from the open field (OF) test were mixed when examining the effect of pair bond formation on behavioral signs of anxiety. There were no significant differences between bonded and non-bonded males for time spent in the inner and outer squares of the apparatus: both groups spent a greater amount of time in the outer squares than in the inner squares, a behavior that is usually indicative of anxiety. Bonded males, however, traveled a greater distance during the test than non-bonded males. Bonded males also frequented the different squares of the apparatus more often-particularly the outer squares. This increase in locomotor activity would suggest that bonded males may have been less anxious. Since there was no difference in time spent between

inner and outer squares for the groups, one of the most common measures of anxiety in this test, the results remain inconclusive as to whether pair bond formation affected anxiety in this species.

Results of the elevated plus maze (EPM) test suggested that pair bonding did not influence stress behaviors. For both trials one and two, there were no differences between bonded and non-bonded males for time spent in the open and closed arms: both groups spent a greater amount of time in the open arms than in the closed arms. There were no differences between groups for latency to enter the open arms. There was a difference in the frequency of entries into the different arms of the apparatus: non-bonded males frequented the open arms more often than the closed arms, and more often than bonded males frequented either arm. Considering that the bonded voles did not spend more time in the open arms, nor exhibit greater locomotor activity, the results of the EPM test indicate that pair bonding did not affect anxiety-like behaviors.

Interestingly, results from the OF test were opposite to what was observed in the EPM test when comparing time spent in the different areas of the apparatus. The OF test is designed to mimic an open field, in which animals that feel secure in their environment (such as the inner squares in this experiment) will explore open areas more often. Prairie voles of both groups spent more time, on average, in the outer squares, suggesting that they may have been more anxious. In the EPM, animals more comfortable with their surroundings are expected to explore the apparatus more, spending more time in the open arms. More anxious animals, on the other hand, should spend the majority of time in the closed arms. In the EPM, prairie voles of both groups spent more time on average in the open arms than in the closed arms, suggesting that they were less anxious and more comfortable with their surroundings. These results were in direct contradiction with the results of the OF test, which suggested that animals were more anxious. Results between tests were also contradictory for locomotor activity, another measure of anxiety in rodents. In the EPM and OF tests, animals with greater locomotor activity are thought of as less anxious. In the OF test, bonded males displayed greater locomotor activity, travelling a greater overall distance

and frequenting different squares more often than non-bonded males. Results of the EPM test were opposite: non-bonded males displayed greater locomotor activity by frequenting the different areas of the apparatus more often than bonded males.

One explanation for these differences in results could simply be the animals' natural history. Prairie voles are commonly referred to in our laboratory as "wall huggers" for their propensity to stay close to the walls when they are released in a closed room, and may be naturally inclined to stay close to the walls within the OF test. In their natural habitat, prairie voles spend the majority of their time in tunnels or exploring the tall grasses, as opposed to being strictly out in the open. Thus, taking natural history and the results of the EPM test into account, the OF test may not be the best paradigm to use as an indicator of anxiety. Alternatively, the fact that animals were exposed to the tests one after another on the same day may have influenced results: bonded males may have exerted more energy being active during the OF test, and may have been less active in the EPM test as a result. It may be beneficial in future experiments to expose animals to behavioral tests on different days, as well as to use more sensitive tests to examine anxiety-like behaviors in prairie voles.

Females were retained until the birth of their first litter to determine when they became pregnant, as this was used to divide males into "bonded" and "non-bonded" groups. One potential limitation in this experiment is that a "partner preference" test, a paradigm commonly used to test for social preferences and long-lasting pair bonds in this species, was not used to further establish the existence of an enduring bond between pairs in this project. A partner preference test is frequently used to test whether a subject spends more time with, and thus "prefers," the familiar partner they have been paired with over an unfamiliar animal. The use of such a test might have provided further information about the different groups, and whether they had truly formed an established pair bond (or not) with their partner. As previously discussed, however, pregnancy following two to three days of pairing should have been enough to establish a robust pair bond.

The results of this experiment showed that pair bonding can influence corticosterone levels over time. The results of the behavioral tests were mixed, but appeared to indicate that pair bonding has minimal, if any, effects on anxiety-like behaviors in prairie voles. As such, the results of this experiment only partially support the original hypothesis: pair bond formation can affect stress hormone levels over time, but not most stress behaviors.



## CHAPTER VIII

### DISCUSSION

#### **Summary of Results**

This project examined the interaction between stress responses and social bonding through two major experiments. The first experiment tested the effect of acute stress in males on female mate choice, an influential factor in social bonding that has not been fully studied in prairie voles. The majority of females in this experiment preferred not to spend time with males that had been exposed to a stressor, instead opting to spend time with a non-stressed control male or with neither male. When females were put through an aversion test that provided the option of spending time with a stressed or non-stressed male, or in an empty cage, there were no significant differences between the choices females made, suggesting that females were not necessarily averse to stressed males. These results indicated that acute stress could potentially have a negative impact on social bonding in prairie voles, as female prairie voles seemed to prefer males that had not been exposed to a stressor.

The second experiment tested the effects of social bonding on stress hormone levels and behavioral indicators of anxiety in prairie voles. Bonded males had lower corticosterone levels than non-bonded males over a 14-day period. There was no difference between groups for time

spent in the inner and outer squares of the open field (OF) test, a behavioral test for anxiety in rodents, although there was a difference between groups for total distance traveled. There were no differences between groups for time in the open arms vs. closed arms in the elevated plus maze (EPM) test, another behavioral test that can be used to measure signs of anxiety, although there was a difference between the frequencies of entries into each arm between groups. Combined results of the behavioral tests suggested that pair bonding did not affect most anxiety-like behaviors.

The results of this project supported the initial hypothesis that acute stress in males would influence mate choice by females. The prediction that acute stress in males would positively influence female mate choice, however, was incorrect: when females made a choice, they preferred males that had not been exposed to a stressor. Acute stress appears to have negatively impacted female mate choice and potentially the development of social bonds. The hypothesis that pair bond formation would influence corticosterone levels over time and behavioral indicators of anxiety was only partially correct: the development of a pair bond appears to influence corticosterone levels over a 14-day period, but does not affect most behavioral signs of anxiety.

### **Significance in the Literature**

The effects of stress on social bonding has been examined before in prairie voles. Earlier studies focused on the effects of an acute stressor, or injection of the stress hormones corticosterone or corticotropin-releasing factor (CRF), on the formation of long-lasting partner preferences. These studies determined that exposure to a stressor or stress hormone could enhance the likelihood of forming a social preference in male prairie voles (Devries et al., 1996; Devries et al., 2002). The current project tested whether the positive effect of stress on bond development in males could potentially be due to female mate choice. The results indicated the opposite: stress in

males appears to negatively affect mate choice by females, as the females in this project seemed to prefer males that had not been exposed to a stressor.

This information adds to our current knowledge of stress and social bonding in prairie voles. We now know that stress in males can negatively affect female preferences, a finding that is important for understanding social bonding, reproduction and even the survival of prairie voles. This knowledge also leads to the conclusion that the effects of stress to increase a male's likelihood of bonding with a female are not due to female mate choice, but to some other factor, such as a male's behavior. A potential theory described in earlier studies for the effects of stress on pair bonding in males was that males were more likely to disperse from the natal nest and find a mate due to stress and aggression during periods of overcrowding (Devries et al., 1996). As female mate choice does not positively influence bonding for male voles, this explanation may require further study in the future.

Previous studies have examined the effects of social isolation on behavioral and neuroendocrine measures for anxiety and even depression in this species. Prairie voles that have experienced chronic social isolation exhibit anhedonia (decrease in sucrose intake and preference) (Grippe et al., 2007). Voles that have undergone chronic social isolation also show behavioral indicators of anxiety in the open field (OT) and elevated plus maze (EPM) tests, and show increased mRNA expression of different hormones, including corticotropin-releasing factor (CRF) (Grippe et al., 2007; Wang et al., 2009). As previously discussed, social relationships can have a positive influence on health through various behavioral, psychological and physiological mechanisms. Positive relationships can also mediate many of the negative effects of stress (Cohen & Wills, 1985; Umberson & Montez, 2010).

For this reason, the effect of pair bond formation on stress hormone levels over time and behavioral measures of anxiety was examined in male prairie voles. The results of this project

indicate that pair bond formation can influence corticosterone levels over time, suggesting that social bonds may protect against the negative consequences of stress by affecting underlying stress hormone levels. This adds to our knowledge of the interactions between stress and social bonding by showing that social bonding can potentially affect the release of the stress hormone corticosterone over time, as well as affecting an animal's direct stress response. Results from this project also provided information on the interactions of stress and social bonding by revealing that pair bond formation does not appear to have much effect on behavioral measures of anxiety.

The results of this project expand upon our knowledge of prairie voles and social bonding. This project has provided information on the influences of stress on a number of different areas. It has increased our knowledge of the effects of stress on mate choice and bonding in this species. It has also added to our knowledge of the effects of social bonds-particularly monogamous pair bonds-on stress in prairie voles and more broadly. The following describes how the current project adds to our knowledge of three major topics: pair bonding and reproduction, mate choice, and the effects of relationships on stress and health.

#### *Pair Bonding and Reproduction*

As discussed, several factors are involved in pair bonding in prairie voles. One of these factors is the timing in which a female becomes pregnant following exposure to an unfamiliar male. Pregnancy needs to be achieved within two to three days of pairing in order for a long-lasting social preference to develop (Curtis, 2010). The mate choice procedure used in this project lasted only two hours. During this short period, the majority of females did not spend even ten minutes with a stressed male. Thus, it is unlikely that females would choose to spend enough time with a stressed male for mating and pregnancy to occur within the short two-to-three day window required for an enduring bond to develop. Stress, then, has the potential to have a profoundly

negative impact on social bonding and reproduction in this species. If female mate choice were the only factor affecting social bonding in this species, stressed males would not reproduce.

The formation of a monogamous bond in prairie voles is also regulated by the neuroendocrine system. A number of hormones are involved in pair bond formation, including oxytocin, arginine vasopressin, dopamine and corticosterone (Carter et al., 1995; Young & Wang, 2004). Previous studies in prairie voles have implicated the hypothalamic-pituitary-adrenal (HPA) axis in pair bonding: exposure to an acute stressor or the hormones corticotropin-releasing factor (CRF) and corticosterone can increase the likelihood of forming a social preference in male prairie voles (Devries et al., 1996). Based on the results of the current project, it does not appear that female mate choice is the reason for an increased likelihood of partner preference formation in males. Females do not prefer stressed males, so it is unlikely that females are the main cause of this phenomenon. The positive effects of stress on preference formation seen in males appear to be caused solely through the male. Thus, the influence of glucocorticoids on the neuroendocrine system may be even more important for pair bonding in males than previously thought.

Glucocorticoids may exert their effects on this species by interacting with other hormones important for social bonding. Vasopressin, for instance, is involved in the stress response and can promote the release of adrenocorticotrophic hormone (ACTH) and production of corticosterone (Whitnall, 1993). Vasopressin content in the central nervous system is also sexually dimorphic in prairie voles, which may explain the different effects of stress on bonding between sexes (Bamshad et al., 1993). Oxytocin has also been put forth as a potential mediator between the effects of stress and pair bonding. Oxytocin may be involved in the production of ACTH and corticosterone during the stress response in rodents (Samson & Mogg, 1990; Devries et al., 1996). Stress can inhibit the release of oxytocin: in female prairie voles, stress exposure may inhibit oxytocin release, which may explain its effect on partner preference formation in females of this species (Devries et al., 1996). The interaction of different hormones during the stress response

may lead to the increased likelihood of partner preference formation in males exposed to stress. As the results of this project indicate that the effect of stress on pair bonding in males is due to mechanisms other than female mate choice, the interaction of these hormones during the stress response appears to be even more important than ever when examining the influence of stress on social bonding in this species.

Devries and colleagues (1996) discussed possible causes for sexual dimorphism on the effects of stress on social preferences in prairie voles. A common stressor for voles in nature is overcrowding - or having a high population density. In such cases, there may not be a need for females to leave the natal nest, as they may be able to reproduce with unfamiliar conspecifics close by. In contrast, males become aggressive and territorial following mating, a reason why a propensity to disperse when exposed to the stress of overcrowding may be beneficial to them. It may be advantageous to study the effects of stress on the overall behavior - affiliative and otherwise - of male prairie voles, as this factor may prove more influential to explain the positive effects of stress on social preferences in male voles than mate choice.

While prairie voles might not be exposed to the exact stressor used in this experiment during natural settings, there are a number of acute stressors voles may experience in the natural world. For example, a primary stressor in the animal kingdom is predation. Prairie voles are significant prey animals, being food for common predators such as birds, small mustelids, feral cats and snakes. As discussed earlier in this project, the size of communal groups varies by season based on the level of predation: nests contain a smaller number of voles in the spring and summer due to high rates of snake predation (Getz et al., 1990; Norrdahl & Korpimaki, 1998; Tamarin, 1985). The results of this project indicate that stress in male prairie voles, at least, can negatively impact mate choice and thus the reproduction and survival of this species. The presence of predators - and any potential increases in them - may then negatively impact populations of

prairie voles not only through predation, but also through a decrease in bonding opportunities due to the increase in stress.

These negative effects can further be compounded by human activity and the environment. Climate change, for example, likely will have a significant effect on the survival and reproduction of different species. Alterations brought on by climate change in species' physiology, phenology and distribution will bring about changes in the relationships between species, including predator-prey interactions. These changes may then lead to alterations in species distribution, numbers and geographic ranges (Hughes, 2000). These changes also may affect predator populations, which could affect vole populations. Changes in vole populations could also affect predator populations in the future. Studies indicate that climate change is already having an effect on rodent populations. Arctic models detailing the effects of climate change show increases in the length of population cycles for lemmings, as well as a reduction in the maximum population density. These changes are harmful to predators, which have evolved to take advantage of prey cycles (Gilg et al., 2009). Cyclic vole populations in Scandinavia have been in significant decline for decades, particularly in winter where there is now a shortened period of protective snow cover (Hornfeldt et al., 2005).

Considering the negative effect of stress on mate choice and, potentially, on reproduction in this prairie voles, the stressors associated with a changing habitat and climate could in themselves have an effect on prairie vole populations. Resources may become scarcer, prospective habitats could decrease in size, or quality, and there may be increases in certain predators. Snakes are a major predator on vole young during the summer season. Steadily increasing temperatures may lead to an increase in snake populations. Not only would these individual stressors have a profound impact on the survival of prairie vole populations on their own, but the added stress could negatively affect the maintenance of such populations.

There are stressors other than those caused by climate change now being introduced to animal - and particularly - vole populations associated with human activity. As listed above, feral cats are a common predator of voles. Most cat populations in the United States were first introduced by humans, either for the purpose of pest control or as pets. It has been suggested that the number of feral cats in this country alone may be over 60 million (Robertson, 2008). The survival of prairie vole colonies will be affected by proximity to these cat populations, and their numbers may be impacted not only through consumption by cats, but by the added stress of being hunted. Increases in human urbanization also interferes with vole colonies as more development interferes with vole habitats. A decrease in such habitats will likely lead to a decrease in the number of prairie voles, and further limited reproductive availability. Stress caused by habitat loss will likely affect mate choice and further reproduction as well. Decreases in vole populations, caused by both loss of resources and potentially stress, will negatively affect predator populations as well.

An increase in the stressors prairie voles face could negatively impact mate choice, decreasing reproductive opportunities. Environmental and human stressors thus have the potential to impact the survival and reproduction of prairie voles, as well as related populations of organisms. Predator populations are largely dependent on prey, and the population cycles of both groups of animals are frequently interrelated. Thus, the effects of stress in this species can be significant to the ecosystem as a whole. The information brought forth through this project on the effects of stress in males on mate choice by females adds to our knowledge about the impact of stress not only on the reproduction and survival of this species, but on the health of other animal populations as a whole.



## *Mate Choice*

“Mate choice” is the tendency of an organism to breed nonrandomly based on a bias toward certain traits in the opposite sex (Kokko et al., 2003). While mate choice has been observed in both sexes, female mate choice is more common for a variety of reasons (Krasnec et al., 2012). Mate choice can provide direct and indirect benefits to the female and offspring: females may prefer traits that will raise the chances for their own survival or fertility, or base preferences on traits that promote the fitness of offspring (Jennions & Petrie, 1997). Mate choice, then, can be a useful method for reproduction, increasing the likelihood of quality individuals to succeed in mating (while decreasing the likelihood of lower-quality individuals to spread their genes). In this way, mate choice can promote the health and survival of a species.

Different factors affecting preferences in prairie voles have been studied prior to this project. Previous research has shown that females normally avoid incest, being more likely to mate with males that are unrelated to them (Getz et al., 2005). Litter composition has also been found to affect female preferences: females typically avoid reproducing with males that came from an all-male litter (Curtis, 2010). Females also show preferences for males with longer anogenital distance, indicating greater fertility (Ophir & delBarco-Trillo, 2007). Lastly, female prairie voles prefer dominant over subordinate males (Shapiro & Dewsbury, 1986). Clearly, several factors can influence mate choice in prairie voles. The effects of male stress on female mate choice, however, had not been thoroughly examined in prairie voles until the current project. Findings from this project have revealed new information on a factor now known to influence preferences in prairie voles, showing that stress can have a negative effect on mate choice in this species.

Stress has already been shown to influence mate choice in different species. Chronic stress in particular has been shown to have negative effects on mate choice throughout the animal

kingdom. Higher levels of baseline glucocorticoids can be indicative of a lower body condition and decreased fitness (Bonier et al., 2009). As body condition and health is negatively correlated with glucocorticoid levels, females frequently display a preference for males with lower glucocorticoid levels. Many of the traits females rely on for preferences in mate choice (bird song quality, ornamentation, vocalization etc.) are indicative of overall health and body condition (Husak & Moore, 2008). As these two factors can be negatively influenced by chronic stress, it is understandable how stress can, in turn, negatively affect mate choice.

Although the current project focused on acute stress rather than chronic stress, the results were similar to studies with different species illustrating the negative effects of male stress on female choice as previously described. Female zebra finches prefer males with lower circulating levels of plasma corticosterone compared to males with higher levels (Roberts et al., 2007), and chronic stress has negatively impacted mate choice in not only finches, but other birds and amphibians by affecting the quality of bird song, ornamentation and vocalizations (Husak & Moore, 2008). This project, then, shows that acute stress may have similar effects on mate choice in animals as chronic stress. The results of this study indicate that if a female in the wild came across a male soon after his exposure to a natural stressor (predators, etc.), she may not be inclined to mate with him-at least, in prairie voles. This finding may extend to other species in the animal kingdom, indicating that exposure to acute stressors in the wild can be harmful to a male's reproductive opportunities.

The findings of this project further illustrate the negative effects of stress - both acute and chronic - on mate choice in the animal kingdom. Female prairie voles appear to prefer males that have not been stressed, just as females of many different species prefer non-stressed males. The results of this project provide further evidence of the detrimental effects of stress on mate choice and reproduction across species. Prairie voles can now be included among the number of animals whose preferences - and reproductive potential - can be negatively influenced by stress.

The “social selection explanation” refers to the idea that individuals that are already healthy are more likely to enter into relationships such as marriage (Shapiro & Keyes, 2008). While this concept was originally proposed while studying human relationships, results from this project indicate that mate choice may come into play regarding this theory. One major factor affecting health is stress. The effects of stress are profoundly negative on mate choice, not just in prairie voles, but in many different species. It is possible that individuals suffering from chronic stress - which has been shown to be correlated with many negative health effects - would be less likely to enter into a relationship. One reason for this could be based on preferences: human women, just like in other species, may prefer less stressed partners. The effects of stress on mate choice could then hold a greater influence on the interaction between health and relationships. If this is true, then stressed individuals (as well as unhealthier individuals in general) would be less likely to enter into relationships due to being rejected as a potential partner more often. Information from this project, then, potentially gives support to the social selection explanation, shedding light on the idea that stressed individuals are less likely to enter into social bonds due to the negative consequences of stress on mate choice, in both prairie voles and potentially other animals, including humans.

This project provides further evidence that stress in males can negatively impact female mate choice in many different species. An increase in stressors in the natural world, as already described, could be detrimental to the reproduction and survival of different species. Human activity has led to a rise in the number of stressors an organism may face. Habitat loss due to urbanization and deforestation, as well changes in the number of predators and environmental alterations caused by climate change, are all stressors many organisms will increasingly face in the future. Not only will these factors decrease survival on their own, but they could potentially reduce reproductive opportunities in different species due to their negative impact on male stress and body condition. An increase in stress in the males of many species could produce negative

consequences for mate choice, decreasing males' reproductive opportunities, which could have a profoundly negative impact on the reproduction and survival of a species as a whole. This project delivers further information suggesting that an increase in environmental changes and stressors can negatively influence mate choice, social bonding and reproduction in different species.

### *The Effect of Social Relationships on Stress and Health*

Individuals with greater social support are generally healthier and live longer, benefitting from reduced chances of a range of different disorders. People in happy marriages, for example, have lower morbidity and mortality (House et. al, 1988; Hold & Lunstad, 2008). Stress - particularly chronic stress - can negatively influence health. Social relationships can reduce the impact of stress (Thoits, 2010). The processes leading to the effects social relationships have on stress are not well understood, although they are believed to involve various psychological, behavioral and physiological mechanisms. The social buffering hypothesis suggests that social support can act as a buffer against many of the negative effects of stress, protecting individuals from many of the negative health consequences (Cohen & Wills, 1985).

Prairie voles are a highly social species and have previously been used in studies focusing on the effects of social bonding on health and different bodily processes. As previously discussed, studies have shown that chronic isolation in prairie voles can lead to neuroendocrine and behavioral changes similar to symptoms of anxiety and depression (Grippe et al., 2007; Grippe et al., 2007). Social bonding also affects autonomic and cardiovascular activity: in one study, pair bonded males had a lower heart rate and higher respiratory sinus arrhythmia than non-bonded males, and showed fewer anxiety-related behaviors in an open field (OF) test (Kenkel et al., 2014). Social isolation following bonding has also been shown to lead to a rise in heart rate, heart rhythm dysregulation and autonomic imbalance in prairie voles (McNeal et al., 2014).

This project examined the influence of social bonding on stress hormone levels and behavioral indicators of anxiety. Previous studies utilizing voles to examine the interaction between social bonds and stress have utilized similar measures to those used in this project (corticosterone measurements, behavioral tests). However, this was the first to examine the effects of pair bond formation on repeated measures of corticosterone across a period of time, as well as the first to utilize fecal instead of plasma corticosterone as a measure for stress levels in prairie voles. It was also the first to examine the effect of bond formation specifically on the results of the elevated plus maze and open field test. The results of this project provide further information on the influence of bond formation on different measures of stress in this species.

The results of this project showed that pair bond formation alters fecal corticosterone levels over time. These results suggest that the formation and maintenance of a pair bond can affect underlying levels of circulating corticosterone. Results from this project also showed that pair bonding was not enough to alter most behavioral patterns observed in the open field and elevated plus maze tests between groups of animals. It appears that, while pair bond formation can affect corticosterone levels over time, it is not sufficient for producing changes in most anxiety-like behaviors in prairie voles.

Previous research has shown that bonded voles can have different stress responses when recovering from a stressor alone vs. with their bonded partner. In one study, females that were isolated following one hour of immobilization had higher circulating levels of corticosterone and more anxiety-like behaviors than females that were allowed to return to their partner (Smith & Wang, 2014). This shows that social bonds can potentially act as a buffer against some of the effects of stress in prairie voles. This is similar to results in studies with other species, showing that the presence of another conspecific, and frequently an animal the individual is bonded to (the mother, etc.) can reduce stress levels during or after exposure to a stressor, as previously discussed (Kikusui et al., 2006).

Results of the current project reveal that social bonding may not only influence the direct stress response, but underlying corticosterone levels as well. This suggests that the buffering effect of social relationships on stress is not only due to the presence of another animal affecting the stress response when a subject is exposed to a stressor, but also to physiological mechanisms associated with stress and health. The buffering effects of social relationships on the negative health consequences of stress may be due to differential effects of social bonds on the HPA axis and other systems associated with stress. If this is true, then these results would lend credence to the social causation hypothesis: social relationships can positively influence health by directly influencing different physiological systems. The results of the current project, then, have important implications for stress and human health. Social relationships may serve to reduce underlying levels of glucocorticoids, hormones that can negatively influence health if chronically elevated. Relationships may also affect other physiological mechanisms, potentially reducing the negative effects of stress on the body, both through attenuating stress responses (as discussed above) and influencing different body systems.

The results of this project indicate that the formation of a monogamous bond does not affect most anxiety-like behaviors when measured in the elevated plus maze (EPM) and open field (OF) tests. This indicates that pair bond formation does not influence all stress responses. It may be that social bonds affect stress through directly mediating the stress response and influencing levels of stress hormones, but not by affecting the behaviors and neurological systems associated with long-term anxiety. More research is needed to fully determine these effects.

The current project added to our knowledge of how social relationships can affect stress through two different measures: corticosterone levels and anxiety-like behaviors. It appears that social relationships can influence fecal corticosterone over time, suggesting that relationships can influence different physiological mechanisms. Results from this project indicate that social

relationships do not influence most stress behaviors in prairie voles, indicating that further study may be needed to determine the effects of relationships on long-term anxiety.

### **Significance in Methods Development**

Mate choice and stress have previously been examined separately in the prairie vole literature. While previous studies have focused on the influence of stress on pair bonding in voles, this was the first to examine the effect of stress on the specific factor of mate choice. In order to ensure that males had been exposed to an adequate acute stressor prior to the mate choice test, a new stress paradigm was developed for this study as well. While resident-intruder tests are a commonly used stressor for rodents, this was the first time, to our knowledge, that a five-minute test followed by 15 minutes of separation by a wire mesh barrier was shown to have the potential to be a useful and important tool for increasing corticosterone levels in prairie voles. Future studies can now utilize this stressor as an accurate and reliable method to expose prairie voles to an acute stressor.

Corticosterone levels have previously been measured in this species for uses in different studies. Previously, however, corticosterone was measured in blood plasma in prairie voles, an invasive method that usually involves terminating voles at each time point when time courses were studied. This project was the first to examine the potential for using fecal corticosterone to measure levels of the stress hormone in this species, particularly across an extended period of time. Through this experiment, it was determined that fecal samples can be used to measure corticosterone, and so can be used for this purpose in future studies. Work from this project has provided a new, noninvasive technique that will allow us to repeatedly measure levels of stress hormone in this species.

## **Limitations**

This experiment used both the elevated plus maze (EPM) test and open field (OF) test to measure anxiety-related behaviors in paired males. As discussed earlier, both of these tests are common behavioral tests for anxiety in rodents. The results of these tests differed from each other in this study. One reason for this is that the OF test may not be an ideal model to examine anxiety behaviors in prairie voles, a potential limitation in this project. Exploratory behavior is commonly used as a measure for anxiety in behavioral tests in rodents (Bailey & Crawley, 2009). The OF apparatus used in this experiment resembled a large, empty box, with little for subjects to explore. Prairie voles in this lab have also been referred to as “wall huggers” for their propensity to stay close to the walls when let loose in a room. Due to this propensity, time spent in the different areas of the apparatus may not be a measure sensitive enough to determine animals’ anxiety in this test. It may be beneficial in future experiments with prairie voles to include objects within the OF apparatus for animals to explore, or use more sensitive behavioral tests, when examining anxiety-related behaviors.

Another potential limitation for this project is that behavioral tests were performed immediately one after another. Following exposure to the OF test, subjects then underwent two trials of the EPM test. Exposure to the OF test prior to the EPM test could have potentially increased anxiety in subjects, which would have impacted the results of the EPM test. However, results show that males did not display an increase in anxiety during the EPM test, and in fact spent more time in the open arms, indicating that they were not anxious. There may, however, have been an influence on locomotor activity: bonded males in the OF test displayed more locomotor activity than non-bonded males, but appeared to be less active in the EPM test than non-bonded males. It may be beneficial in future experiments to expose animals to different behavioral tests on different days.



A final limitation in this project involves separating males into “bonded” and “non-bonded” groups. This study used the female’s parturition status as a way to determine whether or not a male had formed a monogamous pair bond with the female he was paired with. As discussed earlier, when a female becomes pregnant can determine whether or not a robust bond develops between pairs (Curtis, 2010). Previous experiments with prairie voles have also utilized a “partner preference test,” a procedure similar to the mate choice test used in this project, where subjects have the option of spending time with their “partner,” an opposite-sex conspecific they have previously been paired with, or a “stranger,” an unfamiliar conspecific. Such tests have been used to establish whether or not a bond has developed within a pair by determining whether a subject prefers their partner over other conspecifics (Carter et al., 1995). While parturition status should be a sufficient measure to determine whether a bond has developed within a pair, exposing animals to a “partner preference test” at the end of the 14-day experiment would have provided further information on whether animals had indeed developed a bond, and whether choice appeared to influence bonding in these animals.

### **Future Studies**

Males exposed to a stressor or injected with corticosterone are more likely to form long-lasting partner preferences with a novel female. A potential explanation for this finding is that males are more likely to disperse and find a mate after experiencing crowding in the natal nest. This theory, however, has yet to be proven. This project examined whether acute stress exposure in males could influence female mate choice, a factor that could have also been involved in the positive effect of stress on partner preference formation observed in males. The results of this experiment showed that acute stress in males did not have a positive effect on female mate choice. Thus, future experiments may focus on other potential explanations for the effect of stress on partner preferences in males.

One factor that may be examined in the future is male behavior. It may be beneficial to compare behavior between males exposed to a stressor and males that have not been exposed to a stressor, in relation to females as well as apart from them. One aspect of social buffering that has not been given much attention in this project is the tendency for individuals to seek out others following a stressful event. Social support has already been shown to mediate stress levels, and seeking out affiliative partners and social groups can potentially reduce stress following exposure to a stressor (Kikusui et al., 2006; Smith & Wang, 2014). Following stress exposure, females do not display a change in their behavior, although their male partners do. Their partners, in fact, increased their affiliative behavior, establishing contact with the stressed females and involving themselves in social grooming (Smith & Wang, 2014). This illustrates the importance of social behavior in buffering the effects of stress.

Considering the sexual dimorphism in prairie voles involving the effects of stress on pair bond formation, it may be beneficial to examine the effects of stress on social seeking behavior in males in an experiment similar to the one described above. It is possible that stress could influence males to seek out other conspecifics, potentially leading to a reduction in stress levels. Such behavior is common in humans and rats (Smith & Wang, 2012). If this were the case in male prairie voles, then this could potentially explain why males are more likely to form long-term partner preferences following stress exposure. While there were no noticeable increases in affiliative behaviors in either the stressed male or female subject during the mate choice test, it may be beneficial to quantify prosocial behaviors (i.e., approaching, sniffing or grooming) in males as well as potential partners following exposure to a stressor. Since this project focused on mate choice, with females allowed access to two different males and the males restrained to their individual cages, this was not an ideal environment to study “approach” behaviors in either male. Future studies in this area could determine whether stress has an effect on male behavior, and whether this factor explains the effect of stress on partner preference formation in males.

The prairie vole is a social species. When given the option of spending time with another vole or alone, prairie voles will typically choose to spend time with another animal. The partner preference test was designed with this fact in mind: past studies utilizing this paradigm relied on the fact that animals would most likely choose to spend time with one of the two animals presented as opposed to being alone in the empty center cage. The results of this experiment differed in this regard: of the 23 animals that underwent the mate choice procedure, ten “ambivalent” females did not choose to spend ten or more minutes with either male, instead spending significantly more time in the empty, center cage than in either cage that had a male present. These results were unexpected. However, other recent experiments in our lab have yielded similar results. It may be beneficial for future experiments to focus on this group of “ambivalent females,” comparing them with females that made a choice in regards to their behavior, timing for pregnancy, litter size, etc. to determine any factors that may account for the differences in this group of animals.

As discussed earlier, the number of females that had pups at the end of this project differed from expected. Only ten of the 21 females that remained throughout the project had pups within 23-25 days, signifying the development of a lasting partner preference between the male and female pair. It appears that choice, or perhaps stress in the male, might have had some effect on the female’s fertility: of the ten that gave birth, six were females that “chose” a non-stressed male, and the remaining four were ambivalent. However, these numbers were not significant in this project. Future experiments may further examine whether having a choice, and being allowed to stay with the chosen partner, can affect a female’s fertility in this species.

This project compared bonded males with non-bonded males when examining corticosterone levels over time, as well as the results of the OF and EPM tests, using the timing of a pair’s first litter to determine whether animals formed a long-lasting pair bond. Previous research, on the other hand, examined the influence of social bonding on the stress response in

subjects immediately following a stressor, by comparing the physiological responses of bonded individuals who were left alone following stress exposure, and were returned to their partner (Smith & Wang, 2014). However, pregnancy was not used as a measure for social bonding in earlier studies. It may be beneficial in the future to study the influence of social bonding on the stress response following exposure to a stressor utilizing pregnancy as a measure, as it was in this project. It would be useful to examine the effects of pregnancy on social bonding and the actual stress response in the future following exposure to stressors different than the one utilized in this project.

## **Conclusions**

Social relationships can significantly affect health. Marriage in particular has been shown to influence health: studies have shown that individuals in happy marriages have reduced rates of morbidity and mortality (Holt-Lunstad, 2008). Stress is another factor affecting health: while the acute stress response can be important for survival, chronic stress is associated with a number of health problems (McEwen, 2008; Thoits, 2010). There are interactions between stress and social relationships. Stress can negatively impact social bonds: females of different species choose mates with a healthy body condition, which can be negatively affected by chronic stress. Stress has also been shown to negatively influence the health of different relationships in humans, including marriage (Bodenmann et al., 2007; Husak & Moore, 2008). Social relationships, in turn, can serve as a buffer against many of the negative health consequences of stress (Cohen & Wills, 1985).

This project examined the interactions between stress and social relationships. While this study did not involve humans, prairie voles were utilized as an animal model to study social behavior. The results of this project provided further support that stress can negatively influence bonding in different species: results showed that stress in males negatively influenced mate

choice by females. While testing was only performed in prairie voles, this observation adds to the body of literature detailing how stress can have a negative impact on social bonding in various species, particularly for sexual selection. The “social selection” explanation proposes that people who are healthier may be more likely to enter into social relationships, including marriage. While such effects require further examination in humans, it may be that stress – a major factor influencing health – could decrease the likelihood of entering into a relationship (Stack, 1998; Shapiro & Keyes, 2008). If true, this would at least partially explain the observation that people with a high number of relationships (including marriage) enjoy greater health benefits than those with fewer relationships (House et al., 1998).

Alternatively, the “social causation” explanation proposes that relationships may influence health by affecting different physiological mechanisms (Stack, 1998; Shapiro & Keyes, 2008). One way social relationships may affect physiology is through influencing an individual’s stress responses. The “social buffering hypothesis” proposes that relationships can act as a “buffer” against many of the negative health consequences associated with stress (Cohen & Wills, 1985). This project examined how the formation of a monogamous bond might affect different stress measures in male prairie voles. The results showed that pair bond formation can influence stress hormone levels across time, although not stress behaviors. These results may indicate that the formation of different relationships can affect underlying stress hormone levels, suggesting that the formation of social bonds can influence the HPA axis and other physiological mechanisms, buffering against the negative effects of stress by acting directly on different body systems.

The current project added to our understanding of the interactions between stress and social bonding. This project provided evidence to support the hypothesis that stress in males can have an effect on social bonding by negatively influencing female preferences. The reciprocal hypothesis that social bonding would affect stress levels across time as well as behavioral

indicators of anxiety was only partially supported by the results of this experiment: pair bond formation appears to influence stress hormone levels over time, but not stress behaviors. The current project allowed for the development of two new techniques: it is now known that fecal samples can be used to measure corticosterone in prairie voles, and a modified resident-intruder protocol can be used to increase plasma corticosterone in this species. Finally, the current project has provided the potential for several future studies focusing on factors that influence stress and social bonding. Overall, this project has furthered our knowledge about stress, its influences on social bonding, and the effects of social bonding on different stress measures.

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VITA

Stephanie Michele McGlothlin

Candidate for the Degree of

Doctor of Philosophy

Dissertation: INTERACTIONS BETWEEN STRESS RESPONSES AND SOCIAL  
BONDING IN PRAIRIE VOLES

Major Field: Biomedical Sciences

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Biomedical  
Sciences at Oklahoma State University, Tulsa, Oklahoma in July, 2019

Completed the requirements for the Bachelor of Science in Biology  
(Medical/Molecular) at Rogers State University, Claremore, Oklahoma in 2015.

Professional Memberships:

American Physiological Society, 2019

Society for Neuroscience, 2018