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Oxytocin Regulation of Social Buffering Following Stress

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OXYTOCIN REGULATION OF SOCIAL BUFFERING FOLLOWING STRESS

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The work in this dissertation and the success of my academic career is dedicated to Natalie Ann Smith—the epitome of spousal support.

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ABSTRACT

In this dissertation, the neurobiological mechanisms that govern the effects of social buffering on stress were evaluated in female prairie voles (*Microtus ochrogaster*). As reviewed in Chapter 1, social living is beneficial for many species, resulting in increased individual survival and fitness. One factor that seems to lead to such benefits is the anxiolytic effects of social contact with a bonded partner, referred to as social buffering. While stressful life events can enhance the risk of mental disorders, positive social interactions, particularly with a significant other, can propagate good mental health and normal behavioral routines. Still, the functional neural systems that promote these benefits via regulation of the hypothalamic-pituitary-adrenal (HPA) axis recovery are undetermined. Prairie voles engage in and depend on a social environment, including male-female pair-bonds, biparental care, and living in extended families. Like other monogamous mammals, male-female interactions can reduce basal HPA axis activity while promoting stress-reducing neuropeptidergic pathways in prairie voles. In Chapter 2, I evaluated the variability of the behavioral and physiological response as a function of the nature of the stressor in female prairie voles. This is important, as little research has been conducted to study the stress response in voles and determining the responsivity of prairie voles to various stressors will lead to better models of stress in this socially and physiologically unique species. I found stress-specific effects on physiological and behavioral response that varied as a function of the source, intensity, and predictability of the stressor. Furthermore, these data highlight the utility of immobilization within an acute paradigm to characterize the stress response in female prairie voles. Chapter 3 revealed that social buffering from a bonded partner can reverse the aversive effects of immobilization stress on behavior, physiology, and neurochemistry through local activation of the oxytocin (OT) system in the paraventricular nucleus of the hypothalamus (PVN). Recovering from immobilization stress with their bonded partner lead to a reduction in the stress response in female prairie voles. This social buffering by the male partner was accompanied by increased OT release in the PVN. In addition, an intra-PVN OT injection reduced behavioral and physiological responses to immobilization stress whereas an injection of an oxytocin receptor antagonist blocked the effects of the social buffering. This provides evidence for a neural mechanism underlying the social buffering effect from a pair bonded partner in female prairie voles. Finally, in Chapter 4, I discuss these findings and their implications in a general context and suggest future directions for related research.

CHAPTER ONE

GENERAL INTRODUCTION

Adapted from:

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Introduction

Stressful life events are deleterious to mental and physical health, and women seem particularly susceptible. Increased risk of depression is concomitant with the lack or perceived lack of social support following a stressful life event (Dalgard et al., 2006). Social support can ameliorate stress-induced biobehavioral responses and reduce the risk of subsequent psychological disorders (Flannery and Wieman, 1989; Paykel, 1994; Smith et al., 1998b; Cacioppo et al., 2000; Heinrichs et al., 2003; Kikusui et al., 2006). This social buffering effect is dependent on the quality and intensity of the relationship. Therefore, support that comes from deeply rooted social bonds, like romantic social partners, has a greater effect in reducing the negative impacts of stress (e.g., suffering a panic disorder or psychological distress) than support from friends or other relatives (Maulik et al., 2010). Still, the neuroendocrine mechanism underlying social buffering is not well understood, and there are limited modeling of this phenomenon in animal research as less than 3% of mammalian species are monogamous and display social bonding between partners (Kleiman, 1977). In the studies presented in this dissertation, I establish the prairie vole (*Microtus ochrogaster*) as an animal model with which to examine the effects of social buffering on the behavioral and physiological stress response. Prairie voles are socially monogamous rodents that form enduring pair bonds after mating in adulthood, making them an ideal species to use in such studies. While undoubtedly a number of biological pathways contribute to the social buffering of the stress response, the convergence of evidence denotes the regulatory effects of oxytocin (OT) in facilitating social bond-promoting behaviors and their effect on the stress response. Therefore, in the following chapters, I test the hypothesis that OT is a critical neuropeptide system that governs the anxiolytic effects of social

interactions. I hope that the studies presented in this dissertation will serve as a foundation for the future investigation of the neurobiological mechanisms underlying the effects of stress and social buffering.

The Hypothalamic-Pituitary-Adrenal Axis

From Selye (1936; 1950), a stressful stimulus causes disruption to normal homeostatic functions of the body and elicits a physical and physiological response. One of the primary biological pathways that respond to stress is the hypothalamic-pituitary-adrenal (HPA) axis, to the extent that the primary hormones of this pathway – corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormones (ACTH), and glucocorticoids—are referred to as stress hormones. The primary result of HPA axis function is basal release of glucocorticoids and glucocorticoid release during physical or emotional stress. The anterior pituitary secretes ACTH into the circulatory system, and that secretion is regulated by neurotransmitters released by the parvocellular cells of the paraventricular nucleus of the hypothalamus (Tsigos and Chrousos, 2002; Lightman, 2008). The hypothalamus secretes CRH and arginine-vasopressin (AVP) which moves through the hypophyseal portal blood and act on the corticotroph cells of the anterior pituitary increasing pro-opiomelanocortin (POMC) transcription and releasing ACTH from the anterior pituitary (Vale et al., 1981; Aguilera et al., 1983; Smith et al., 1998a; Timpl et al., 1998). CRH seems to be the most potent factor in releasing ACTH (Bilezikjian and Vale, 1987). In fact, even though AVP may lead to additional release of ACTH in concert with CRH, it has little effect alone and seems to be primarily responsible with feedback to the hypothalamus to elevate CRH release (Lamberts et al., 1984; Oosterom et al., 1984). During non-stressed periods, the hypothalamus secretes CRH and AVP in pulses and follows circadian rhythms resulting in basal levels of ACTH and glucocorticoids, in turn. During acute stressful periods, there is a rapid increase in the expression of the immediate-early gene product c-Fos and an increased secretary pulses of CRH and AVP causing elevated ACTH and glucocorticoids (Holmes et al., 1986; Lenz et al., 1987; Bonaz and Rivest, 1998). During chronic stressful periods, CRH expression is damped, but AVP secretion is elevated (Fendler et al., 1964; Hashimoto et al., 1988; Hauger et al., 1988; de Goeij et al., 1991; De Goeij et al., 1992).

The main site in which CRH from the hypothalamus acts is the anterior pituitary resulting in a release of ACTH into the blood stream. However, the major site that ACTH acts on is not in the brain but rather the adrenal cortex (Ontjes et al., 1977). The release and resulting levels of ACTH in the blood act on the adrenal cortex to release glucocorticoids in time of acute or chronic stress or non-stress. Ontjes noted that ACTH actions on the middle zona fasciculata of the adrenal cortex increase the number of low-density lipoprotein receptor resulting in an increase of cholesterol. In addition, ACTH stimulates the cleavage of the side-chain of cholesterol producing pregnenolone, the first step that converts cholesterol into cortisol. Therefore, cortisol production from the adrenal cortex can be promoted and enhanced by the function of the hypothalamus and pituitary.

Glucocorticoids (e.g., cortisol and corticosteroids) major function is homeostasis of the body and reactivity to stress (Reul and de Kloet, 1985; Pratt, 1990; Tsigos and Chrousos, 2002). Glucocorticoids inhibit immune and inflammatory pathways by binding to cytoplasmic receptors, translocating into the nucleus, and interacting with glucocorticoid responsive elements on DNA as well as inhibiting immediate-early gene product (e.g., c-jun and Fos) and NF-kB, promoters of immune cell growth (Scheinman et al., 1995). Glucocorticoids can also inhibit levels of pituitary growth hormonee, gonadotrophin and thyrotrophin secretion (Casanueva et al., 1990; Chrousos, 2000). Along with glucocorticoids role during stress responsivity, there is a feedback mechanism in which glucocorticoids can reduce HPA axis activity at the level of either the hypothalamus or the pituitary (Tasker, 2006). Glucocorticoids can reduces the secretion and expression of CRH from the paraventricular nucleus of the hypothalamus (Young and Akil, 1985; Kovács et al., 1986; Hu et al., 1992; Légrádi et al., 1997), through glucocorticoid receptors (Covenas et al., 1993; Albeck et al., 1994), or act directly on the pituitary preventing further activation of the anterior pituitary or the adrenal cortex (Bilezikjian and Vale, 1983; Fremeau et al., 1986; Bilezikjian and Vale, 1987; Eberwine et al., 1987). The inhibition of ACTH production by glucocorticoids limits the catabolic, antireproductive, and immunosuppressive response of tissues to these hormones (Charmandari et al., 2004). Thus, the HPA axis has a cascading signal that culminates in the production and release of glucocorticoids. This end-product causes a multi-system response to stress. In addition, the actions of glucocorticoids on the hypothalamus and pituitary are to inhibit further release of CRH and ACTH, respectively, causing a two-point negative feedback loop.

The innate function of the HPA axis is to arouse the system to changes in the environment, which can be adaptive during an acute response (e.g., mobilizing energy reserves during a predator attack: Sapolsky, 1998). Stress-induced mental pathologies are usually the result of repeated or prolonged stress, which is characteristic to stress derived from the social environment. The social stress-induced activation of the HPA axis seems to be a major pathway underlying many of these stress-related psychopathologies. Here, I will review the evidence in humans to establish the relationship between social stress and mental diseases and disorders, focusing on depression, anxiety disorders, and drug addiction. Several animal models of social stress that utilize ethologically relevant conditions and species known to form strong social bonds will be discussed to outline the stress-induced activation of the HPA axis as a major biological pathway that underlying the manifestation of such pathologies. In contrast, social relationships can buffer against stress-induced effects on depression, anxiety, and drug addiction via an OT-mediated pathway. Therefore, I will discuss the effects that OT has on alleviating and preventing the activation of the HPA axis and subsequent mental health problems.

The Negative Effects of Stress: Human Mental Health

Social stress in adulthood can be debilitating to mental health and well-being, and no adult relationship is more important in humans than the one between significant others, which typically manifest as a strong social bond, stable social environment, an integral aspect of human social behavior. This close social relationship has been implicated in psychological health and well-being. Therefore, it is not surprising that when this social bond is lacking or maladaptive, a number of stress-related mental pathologies may manifest. For example, compared to their married counterparts[1], single men and women who have never married not only report lower positive relations but also higher depression and hostility scores, more feelings of loneliness, and lower global happiness, life satisfaction, and self-acceptance scores (Marks and Lambert, 1998; Waite and Hughes, 1999; Pinsof, 2002; Mirowsky and Ross, 2003; Williams, 2003; Steptoe et al., 2004), all markers of increased psychological distress. Social loss and spousal bereavement also increase the risk for mental disease and disorders (Barrett, 2000; Carr et al., 2000; Carr et

[1] Here, I focus on marital status as marriage is commonly used as an index of pair-bonding in humans (Pinsof, 2002). Furthermore, committed, intimate adult relationships significantly impact mental health, as they are one of the most significant relationships in humans.

al., 2001; Wade and Pevalin, 2004). Notably, the risk for depression and anxiety can manifest before the death of a spouse (Williams et al., 2008) and last for decades afterward (Carnelley et al., 2006). Even temporary social separation can result in an increased perception of anxiety in men (Diamond et al., 2008). However, widowhood may be particularly stressful as it may be preceded by a period in which the deceased spouse was in poor health, reducing a source of social support and creating a source of stress. It should be noted that the negative effects of social isolation on mental health are not limited to heterosexual couples. Interestingly, a recent study noted that single gays and lesbians reported higher psychological distress (i.e., internalized homophobia, depressive symptoms, and stress) and a lower sense of well-being (i.e., the presence of meaning in life) than individuals in committed or legally-recognized same-sex relationships (Riggle et al., 2010). The quality and intimacy associated with heterosexual marriages are often not distinguishable from that which is observed in committed same-sex couples (Roisman et al., 2008). This may explain the fact that the lack of a strong social tie in humans that usually manifest in adults seems universally detrimental to mental health.

Getting married tends to improve mental health, reducing the risk of depression, anxiety, and substance abuse (Horwitz and White, 1991; Marks and Lambert, 1998; Simon, 2002). However, psychological distress can be increased in married individuals if the marital quality is poor due to strain, conflict, or abuse. The negative effects of marital strain or conflict may be more salient than the positive effects of marriage as individuals tend to dwell more on negative than positive encounters, repeatedly replaying them mentally (Taylor, 1991). Therefore, the tendency to worry over stressful social interactions could further impair psychological well-being in people during periods of marital conflict. Poor marital quality can culminate in divorce, and studies that examine marital change due to separation or divorce note a marked increase in depressive affect (Marks and Lambert, 1998; Simon, 2002; Wade and Pevalin, 2004). Some of these deficits may be attributable to social selection as healthy, well-adjusted individuals usually are attractive partners. Nevertheless, there is evidence that the lack of a significant adult social bond associated with a marriage or committed same-sex relationship directly leads to poorer mental health (e.g., Johnson and Wu, 2002). Divorce is a process beginning well before the discrete legal event, and therefore, the declines in psychological well-being associated with divorce can be due to the conflict within the marriage that precedes the divorce. Furthermore, even when divorce is desired, it can be stressful, and the social disruption associated with

divorce may initiate a period of chronic stress associated with becoming a single parent, losing a source of emotional support, continued conflict with an ex-spouse, and economical decline (Williams and Umberson, 2004; reviewed in Amato, 2000).

Neurobiology of Stress: HPA Axis

One of the major pathways in which social stress induces pathologies and behavioral consequences is the HPA axis. Numerous experiments in humans indicate an association between stress-induced activation of the HPA axis, particularly the release of its end product glucocorticoids, and increased psychological distress that can manifest in mental pathologies. For example, in traditional laboratory psychosocial stressors (e.g., Trier Social Stress Test) and non-laboratory social stressors (e.g., temporary separation from a marital partner), the stress-induced cortisol response is associated with an increased perception of anxiety (Ditzen et al., 2007; Robles, 2007; Diamond et al., 2008). In clinical research, individuals with depression, anxiety, fear, and panic disorders often have dysregulated HPA axis activity, raised basal plasma cortisol, and more intense ACTH and cortisol responses to stress (Rothschild et al., 1989; Tse and Bond, 2004; Vreeburg et al., 2009; Vreeburg et al., 2010; reviewed by Papathanassoglou et al., 2010). A number of animal models of social stress have been developed to better understand the effects and function of the HPA axis and its stress hormones on psychopathologies.

Prairie Voles and Stress

In several socially monogamous New World rodents and primates, disruption to or absence of the close social bond that forms in male-female breeding pairs has been used to assess the HPA axis-mediated effects of social stress on mental pathologies. For example, individual housing can lead to an increased HPA axis and depression- and anxiety-like behavioral response to acute stressors (e.g., open field, elevated plus maze, forced swim, and sucrose preference tests) compared to social housing in monogamous rodents such as prairie voles (*M. ochrogaster*) (Stowe et al., 2005; Grippo et al., 2007a; Grippo et al., 2007b; Grippo et al., 2007c; Grippo et al., 2008; Grippo et al., 2009; Pournajafi-Nazarloo et al., 2009; Toth and Neumann, 2013). Furthermore, pair-bonded male prairie voles have elevated basal corticosterone levels and an increased depression-like response to acute psychological stressors (e.g., forced swim or tail

suspension test) after experiencing a permanent, long-term separation from their female partner, but this is not true when males are permanently separated from a familiar male (Bosch et al., 2009). Inhibition of the stress-induced activation of the HPA axis via a nonselective CRH receptor antagonist or selective CRH-1 or CRH-2 receptor antagonists can eliminate the depression-like response to acute psychological stressors that is induced by the loss of a female partner in pair-bonded male prairie voles. It seems that disruption to or the absence of an adult social bond can impair mental health via the activation of the HPA axis.

Interestingly, the response to social isolation or separation from familiar conspecifics may depend upon the intensity of existing affiliative bonds between group members. In prairie voles, social separation from a same-sex conspecific does not seem to alter basal corticosterone concentrations, even during periods of isolation (Grippo et al., 2007b; Pournajafi-Nazarloo et al., 2009). However, basal corticosterone levels in circulation are higher in prairie voles housed with a same-sex conspecific compared to pair-bonded voles (Campbell et al., 2009). Further, exposing unpaired male and female prairie voles to an opposite-sex conspecific reduces basal corticosterone levels, but exposure to a same-sex conspecific does not (DeVries et al., 1995; Carter et al., 1997; DeVries et al., 1997b). Thus, it seems that the stress-induced activation of the HPA axis and subsequent impairment of an individual's psychological well-being is dependent on the type of bond that is absence or disrupted. Thus, in humans and socially monogamous species in which the significant relationship is between romantic or committed partners, deficits to this bond seem to be particularly damaging to mental health.

In some cases, HPA axis function, can suppress the formation of pair bonds. For example, under short-term cohabitation (1 h), female prairie voles do not display a partner preference, a selective preference to interact with a familiar male over an unfamiliar male used as a laboratory index of the formation of a pair-bond (Aragona and Wang, 2004). In contrast, a partner preference is displayed under long-term cohabitation (24 h or longer). However, if female prairie voles are adrenalectomized (ADX) before a short-term cohabitation with a male, they display a partner preference, while corticosterone injections can inhibit partner preference formation in ADX females (DeVries et al., 1995). Furthermore, partner preference formation after a long-term cohabitation with a male is impaired in intact females exposed to a psychological stressor (e.g., forced swim test) or injected with corticosterone, but not ADX

females (DeVries et al., 1995; 1996). Thus, impairing bond formation may be another mechanism through which psychopathologies may arise via a glucocorticoid-mediated pathway.

New World Monkeys and Stress

The absence of a pair-bonded partner also results in more anxiety-like behaviors and excited HPA axis function in socially monogamous primates such as marmoset (family: *Callitrichidae*) and titi monkeys (genus: *Callicebus*). Social isolation or separation from a bonded partner in marmosets increases basal and stress-induced cortisol response and anxiety-like and social behaviors (Johnson et al., 1996; Smith and French, 1997; Smith et al., 1998b; Shepherd and French, 1999; Gerber et al., 2002; Gerber and Schnell, 2004; Rukstalis and French, 2005; French et al., 2007). These effects are similar in titi monkeys (Mendoza and Mason, 1986a; Mendoza and Mason, 1986b; Hennessy et al., 1995; Fernandez-Duque et al., 1997). If the stress-induced activation of the HPA axis associated with separation from a long-term pair-bonded partner is inhibited by a CRH-1 receptor antagonist (e.g., antalarmin), the magnitude of the cortisol response and display of vocal distress and agitated- or anxiety-related behaviors are significantly augmented in marmosets (French et al., 2007). Thus, psychological distress associated with social separation seems to be mediated by the activation of the HPA axis.

Interestingly, social isolation prior to the establishment of a new social pair increases proximity seeking behavior in marmosets, and this increase in proximity seeking behavior is associated with higher cortisol levels (Smith et al., 2011). As adult social bonds are a fundamental aspect of the social environment in humans and socially monogamous non-human primates and rodents, the absence of or interference with this bond may represent a major disturbance to the normal function and state of these animals leading to the activation of the HPA axis. If the stressful social environment persists, the HPA axis activity seems to become dysregulated leading to psychological and behavioral pathologies. In contrast, attachment behaviors and social contact increases an individual's sense of security, particularly in stressful periods in humans (Shear and Shair, 2005; Ravitz et al., 2010), and decreases the basal and stress-induced HPA axis activity in marmosets (Smith and French, 1997; Smith et al., 1998b; Smith et al., 2011). Therefore, the increased proximity seeking behavior may be an adaptive response to social isolation to minimize the detrimental effects of this type of social stress. The

formation and maintenance of social bonds may result, in part, from the psychological distress associated with bond disruption or absence and the anxiolytic benefits of social interactions.

The Advantageous Effects of Social Buffering

Stress in the social environment due to the lack of close social relationships in adulthood is maladaptive to mental health and well-being; however, social support, particularly from close social relationships such as a committed or pair-bonded partner, can reduce stress-induced behavioral and psychological manifestations (Flannery and Wieman, 1989; Smith et al., 1998b; Heinrichs et al., 2003; Maulik et al., 2010; recently reviewed in Insel, 2010; Karelina and DeVries, 2011). For example, the occurrence of depression is significantly lowered in previously single men and women that marry for the first-time (Marks and Lambert, 1998). Substance abuse and the risk of relapse is markedly lower in individuals in committed, stable relationships in humans (Kosten et al., 1987). One reason social relationships and affiliative interactions may improve mental health is by reducing the HPA axis activity and psychological distress that is associated with a stressful life event or condition. For example, physical contact or verbal support from a committed partner or best friend can significantly lower cortisol concentrations during a psychologically stressful period compared to support from a stranger or no support in humans (Kirschbaum et al., 1995; Heinrichs et al., 2003). Social contact or housing also lowers basal and stress-induced glucocorticoid levels in animals that establish adult social bonds like prairie voles, marmosets, and titis (see "Neurobiology of Social Stress: HPA Axis") as well as California and cactus mice (*Peromyscus californicus* and *P. eremicus*: Glasper and DeVries, 2005; Chauke et al., 2011), guinea pigs (*Cavia aperea f. porcellus*: Sachser et al., 1998; Kaiser et al., 2003; Adrian et al., 2008; Hennessy et al., 2008), and dwarf hamsters (*Phodopus sungorus* and *P. campbelli*: Castro and Matt, 1997; Reburn and Wynne-Edwards, 1999; Detillion et al., 2004). Interestingly, these social relationships both promote and are regulated by the OT system (reviewed in Young and Wang, 2004; Young et al., 2005). In addition, OT can directly attenuate the activation of the HPA axis and is associated with reducing the stress-induced psychological health risks. Thus, it is completely logical to speculate that OT may mediate the social buffering of the stress response and subsequent psychological distress.

Neurobiology of Social Buffering: Oxytocin

Evidence for an OT-mediated mechanism of social buffering on stress includes research in humans and gregarious animals. OT appears to function as an anxiolytic, suppressing HPA axis function during periods of stress. OT released in the hypothalamus or exogenously administered attenuates activation of the HPA axis and reduces depression- and anxiety-like behavior during psychological stress (Legros et al., 1987; Windle et al., 1997; Petersson et al., 1999; Neumann et al., 2000b; Heinrichs et al., 2003; Windle et al., 2004; Parker et al., 2005; Ditzen et al., 2009; Zheng et al., 2010; Linnen et al., 2011). For example, OT is released in the PVN of male rats during mating with a receptive female and proceeded by reduced anxiety-like behavior during psychological stress, lasting at least 4 h after mating (Waldherr and Neumann, 2007). In addition, intracerebroventricular (icv) administration of OT can reverse stress-induced social avoidance to follow social defeat in rats and mice (Lukas et al., 2011). Inhibition of OT action in brain regions that release OT during psychological stress (e.g., PVN: Bosch et al., 2004; central amygdala: Ebner et al., 2004) via site-specific or icv administration of an oxytocin receptor (OTR) antagonist can attenuate the stress-induced physiological and behavioral response (Neumann et al., 2000b; Ebner et al., 2004).

In humans, social relationships are also associated with activity of the OT system. For example, higher plasma OT levels are associated with more positive communication and physical contact between married couples (Turner et al., 1999; Grewen et al., 2005; Holt-Lunstad et al., 2008; Gouin et al., 2010). When interaction with a social partner is associated with elevated plasma OT, it seems that blood pressure and heart rate is lowered (Light et al., 2005). In addition, intranasal OT administrations can reduce the cortisol response during couple conflict and promote positive communication (Ditzen et al., 2009). Further, social support attenuates the cortisol and subjective response (e.g., increases calmness and decreases anxiety) to psychosocial stress in humans, and intranasal OT treatments can potentiate these effects (Heinrichs et al., 2003; Quirin et al., 2011). Conversely, OT may serve as an index of relationship distress as women with perceived poor relationships or social networks can have elevated plasma OT concentrations (Taylor et al., 2006; Taylor et al., 2010). Thus, as OT seems to be an impetus for social interactions, OT activity during relationship distress may facilitate social reconciliation or integration as an active coping mechanism. While there is debate about the delivery route of

intranasal OT or whether plasma OT reflects central activity, the data in humans evaluating the dynamics between OT, prosocial interactions, and the stress response seems to be consistent with more direct evidence from animal models. Therefore, it could be hypothesized that while social stress can promote psychological distress via the activation of the HPA axis, social support can attenuate these effects by marginalizing the HPA axis activity via activation of the central OT system.

Several recent studies have provided more evidence that directly supports this hypothesis. Most, if not all, mammalian species exist in an highly complex social environment, and social living often are critical to the fundamental aspects surrounding fitness, including reproductive success, predatory response, territory and resource allocation and defense, and offspring care (reviewed in Kleiman, 1977; Neumann, 2009). In species that display highly developed social structures, social interactions persist throughout daily life and can modulate the response to various stimuli including stress. Therefore, social stress can have profound effects on the mental state of humans and gregarious animals, particularly in the absence of social bonds. In several socially monogamous (e.g., hamsters and prairie voles) and gregarious (e.g., rats and mice) rodent species, social isolation is met with activation of the HPA axis that promotes depression- and anxiety-like behaviors (Detillion et al., 2004; Waldherr and Neumann, 2007; Grippo et al., 2009; Norman et al., 2010; Toth and Neumann, 2013). However, if OT is administered during social isolation, the increased basal and stress-induced activation of the HPA axis and the subsequent consequences to depression- and anxiety-like behavior are alleviated (Detillion et al., 2004; Grippo et al., 2009; Norman et al., 2010). For example, female prairie voles that are chronically isolated display higher depression-like behavior in response to psychological stress and symptoms of anhedonia (e.g., a diminished sucrose preference) compared to a period of social housing; however, if OT is administered during social isolation, these depression-like symptoms are not observed (Grippo et al., 2009). Therefore, the negative impact of social isolation on the stress pathway and psychological or behavioral pathologies can be attenuated by OT action. In two studies, the beneficial effects of social interactions were diminished by the inhibition of OT action by an OTR antagonist. Specifically, while mating can reduce anxiety-like behavior during psychological stress in male rats, the social buffering effect of mating can be inhibited by a central infusion of an OTR antagonist (Waldherr and Neumann, 2007). In addition, social housing can prevent the development of depression-like behavior during

psychological stress in mice with chronic pain, yet blockade of the receptor-mediated action of endogenous OT inhibits the attenuation of depression-like behavior in socially-housed mice (Norman et al., 2010). These studies support the contention that the mental health-promoting effects of social relationship are regulated by the central OT system.

The Prairie Vole: A Model for Stress and Social Buffering

Prairie voles (*M. ochrogaster*) are not commonly used in stress research, and yet, there are several unique features of vole physiology and social system that make them a promising rodent model of stress. First, prairie voles exhibit high basal plasma corticosterone levels[2], 5 to 10 times higher than laboratory rats and mice (Taymans et al., 1997). However, voles do not display any of the common aversive consequences associated with chronic hypercortisolism. One reason for this non-pathologic hypercortisolism may be that the corticosteroid signal seems to be reduced by elevated corticosteroid binding globulin—though unbound CORT remains high in voles—and decreased glucocorticoid receptor affinity in central and peripheral tissue (Taymans et al., 1997; Hastings et al., 1999). Despite being glucocorticoid resistant, the prairie vole HPA axis is still responsive to circadian cues (Taymans et al., 1997), various stressors (e.g., DeVries et al., 1996; Taymans et al., 1997; Liu et al., 2001; Grippo et al., 2008; Bosch et al., 2009), and social cues (DeVries et al., 1995; Carter et al., 1997; DeVries et al., 1997b; Grippo et al., 2009). Therefore, beyond being an interesting model of glucocorticoid resistance, stress research with prairie voles may serve to better understand the factors that influence variability in the stress response.

Second, the prairie vole social system facilitates the study of the social environment on stress physiology and behavior. Prairie voles are gregarious mammals and live in a fundamentally social environment, including male-female pair bonds, biparental care, and extended families (Carter et al., 1995a; Keverne and Curley, 2004). Among these social relationships, none is as important as the male-female pair bond. Female prairie voles form long-

[2] As already indicated, glucocorticoids, such as corticosterone, are the end-product of the HPA axis and released from the adrenal cortex. Glucocorticoids have two receptor classes mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), with a higher affinity for MR (Reul and De Kloet, 1985). There is an enormous amount of data from numerous studies that indicate that glucocorticoids and their receptor activity play a central role in the prevalence and manifestation of stress-induced physiological, psychological, immunological, and behavioral changes that can culminate the manifestation of psychopathologies.

term selective social bonds (i.e., pair bonds) after mating or continuous cohabitation with a male conspecific, defined by a social preference for a familiar mate over an opposite-sex conspecific, intruder-directed aggression, and bi-parental care (Insel and Hulihan, 1995; Insel and Young, 2001; Aragona and Wang, 2004). Moreover, pair bonding has a major influence on the stress system in female prairie voles. A number of recent studies have denoted that the absence of social contact in prairie voles can promote disruption to normal HPA axis activity and behavioral routines that mimic symptomatology of depression and anxiety disorders in humans (see "Neurobiology of Stress: HPA axis"). In addition, prairie vole pair bonding facilitates a significant reduction in basal HPA axis activity (Carter et al., 1995b; DeVries et al., 1995; Carter et al., 1997; DeVries et al., 1997b), while separation from a bonded-partner can increase plasma corticosterone concentrations (Bosch et al., 2009). As female prairie voles will only develop a strong social bond towards a male partner but will cohabit with other females before forming a social pair with a male, voles provide an animal model to evaluate the effect that social support from different sources has on the stress response. Moreover, as more emphasis is given to understand how different facets of the vole social system influences the stress response, knowledge about how these same factors regulate human stress may be gleaned. Finally, the neuropeptide OT plays an essential role in pair bonding in female prairie voles. For example, the preference for a social partner can be enhanced by icv injections of OT and inhibited by OTR antagonist (Cho et al., 1999). In addition, positive social interactions promote central OT release in female prairie voles (Ross et al., 2009). This is important as OT release in the PVN—which also contains CRHergic neurons that project to the pituitary to control ACTH secretion—attenuates stress reactivity (see "Neurobiology of Social Buffering: Oxytocin"). Therefore, prairie voles may serve as a unique model system to study the anxiolytic effects of interacting with a social partner following a stressful event and the potential mediating role of OT.

CHAPTER TWO

BEHAVIORAL AND PHYSIOLOGICAL RESPONSES OF FEMALE PRAIRIE VOLES (*MICROTUS OCHROGASTER*) TO VARIOUS STRESSFUL CONDITIONS

Adapted from:

Smith, A.S., Lieberwirth, C., and Wang, Z., (in press). Behavioral and physiological responses of female prairie voles (*Microtus ochrogaster*) to various stressful conditions. *Stress*.

Introduction

Stressful events are common aspects of life and can originate from a number of environmental sources, including psychological, social, and physical. Stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis involves a cascade of physiological changes that have been associated with the stress-induced effects on emotional processing (Erickson et al., 2003), normal behavioral routines (Blanchard et al., 2001; DeVries, 2002), and mental health (Young, 2004; Smith and Wang, 2012). The risk for such disruptions to normal homeostatic function may be associated with the context or nature of the stressor as well as how such experiences affect the HPA response to subsequent stressors (Armario, 2006).

Hennessy and colleagues (1979) noted that plasma corticosterone levels rise in response to exposure to a novel environment in rats, and this corticosterone response depended on the duration of exposure as well as the degree of environmental unfamiliarity. In succeeding studies, the stress intensity and previous stress experience have also been implicated to modulate the HPA axis stress response in humans and rats (Armario et al., 1996; García et al., 2000). Thus, characteristics associated with a stressor can dictate the responsiveness of the HPA axis, particularly the rise in plasma glucocorticoids. In addition, the stress-induced glucocorticoid response can lead to increased psychological distress, influencing the psychological state in humans and behavioral routines in animals. For example, the stress-induced cortisol response in humans is associated with an increased perception of anxiety in individuals exposed to psychosocial stress in the laboratory (e.g., Trier Social Stress Test) and in life (e.g., temporary separation from a marital partner) (Ditzen et al., 2007; Robles, 2007; Diamond et al., 2008). Calvo and Volosin (2001) noted that administration of a glucocorticoid synthesis inhibitor (e.g.,

metyrapone) or adrenalectomy can eliminate the anxiogenic effects of restraint stress on anxiety-like behavior in rats. In the same study, an injection of corticosterone, a mineralocorticoid receptor agonist (e.g., deoxycorticosterone), or a glucocorticoid agonist (e.g., dexamethasone) restored the anxiogenic effects of stress. Thus, the action of glucocorticoids and their receptors are sufficient and necessary to cause some the behavioral and physiological effects that are associated with acute stress. However, such physiological activation seems dependent on the context of the stressful event. These data highlight the importance of understanding stress-related factors that modulate the behavioral and physiological response toward a primary stressor as well as the adaptability of the stress system toward subsequent stressors.

Prairie voles (*Microtus ochrogaster*) live in a fundamentally social environment, including male-female pair-bonds, biparental caregiving, and extended families, but are also highly territorial (Getz et al., 1981; Carter et al., 1995a; Keverne and Curley, 2004). Vole behavior and physiology is acutely attuned to alterations to the social environment or other environmental cues. In fact, a number of recent studies have denoted that the absence of social contact in prairie voles can promote a disruption to normal HPA axis activity and behavioral routines that mimic symptomatology of depression and anxiety disorders in humans (Stowe et al., 2005; Grippo et al., 2007a; Grippo et al., 2007b; Grippo et al., 2007c; Grippo et al., 2008; Grippo et al., 2009; Pournajafi-Nazarloo et al., 2009; Lieberwirth et al., 2012). In addition, prairie vole pair bonding facilitates a significant reduction in basal HPA axis activity (DeVries et al., 1995; Carter et al., 1997; DeVries et al., 1997b), while separation from a bonded-partner can increase plasma corticosterone concentrations (Bosch et al., 2009). Furthermore, despite being glucocorticoid resistant (Taymans et al., 1997; Hastings et al., 1999), the prairie vole HPA axis is still responsive to various stressors (e.g., DeVries et al., 1996; Taymans et al., 1997; Liu et al., 2001; Grippo et al., 2008; Bosch et al., 2009), social cues (reviewed in Smith and Wang, 2012), and circadian cues (Taymans et al., 1997). However, little research has been done to determine whether the behavioral and physiological response in prairie voles varies as a function of the nature of the stressor. This is important as determining the responsivity of prairie voles to various stressors will lead to better models of stress in this socially and physiologically unique species.

Thus, the current study examined the characteristics of stress, including source and intensity as well as context of previous stress experience to a homotypic stressor, on the response of the HPA axis and subsequent behavioral manifestations during a secondary stressor (the

elevated plus maze, EPM, test). Socially housed female prairie voles (*Microtus ochrogaster*) were exposed to various acute psychological and social stressors (Experiment 1) or were exposed to a homotypic stressor (immobilization) for 1, 3, or 7 days in a predictable daily schedule or an unpredictable varying schedule (Experiment 2). In Experiment 1, I utilized two psychological stressors that have been demonstrated to vary by intensity while still provoking a biobehavioral response in rodents (i.e., mild stress: environmental novelty; severe stress: immobilization stress). In addition, I exposed female prairie voles to a resident-intruder paradigm in which they entered the home cage of an aggressive same-sex conspecific to induce social defeat. I utilized pair-bonded female prairie voles as the aggressive conspecifics as cohabitation with a male will facilitate territorial behavior in female prairie voles (Getz et al., 1981), and they will display aggressive behavior toward an intruder in a resident-intruder confrontation. I utilized two separate social defeat paradigms that varied in the length of physical confrontation with the aggressive conspecific and ability of the defeated intruder to withdrawal from continued confrontation. I predicted that the behavioral and physiological response to each of these acute stressors will depend on the source and intensity of the stressor. In Experiment 2, I focused on whether the stress response in female prairie voles was affected by the predictability of a repeated homotypic stressor. While repeated exposures to immobilization stress can lead to habituation to this stressor when it is predictable (Martí and Armario, 1997; Girotti et al., 2006; Gagliano et al., 2008; Rabasa et al., 2011), it has been demonstrated that an irregular or unpredictable schedule of immobilization stress does not desensitize, and can even augment, the stress response in male rats (Quirce et al., 1981). Therefore, I predicted that female prairie voles would adapt to repeated exposures of immobilization stress as a function of predictability, with predictable immobilization stress producing a habituating effect and unpredictable immobilization stress augmenting the biobehavioral stress response.

Methods

Subjects

Subjects were captive-bred female prairie voles (*M. ochrogaster*) descended from populations in southern Illinois. Subjects were weaned at 21 days of age and then housed in same-sex age-matched pairs in plexiglass cages (29 L × 18 W × 13 H cm) containing cedar chip

bedding. Food and water were provided *ad libitum*. Colony rooms were maintained at 21±1 °C with a 14L:10D photoperiod (lights on at 0700 h). Before the start of this study, all female subjects were sexually naïve and of adult age (between 90 and 120 days of age). Two weeks prior to the first stress exposure, female subjects were housed with an unfamiliar, unrelated, vasectomized adult male. Experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Florida State University.

Stress Paradigms

Numerous environmental stimuli can be defined as stressors. The current study utilized four stress paradigms that vary in the nature of the source, predominantly psychological or social stress, and the intensity or severity, low or high. The stressors employed in this study included environmental novelty (low intensity, psychological stress), immobilization (high intensity, psychological stress), and social defeat with a brief (15 min; low intensity, social stress) or prolonged (30 min; high intensity, social stress) physical confrontation. In the novel environment paradigm, subjects were placed into an open field arena (56 L × 56 W × 20 H cm) for 60 min. In the immobilization (plastic mesh) paradigm, subjects were exposed to 60 min of immobilization in restraint tubes constructed from PVC pipes (10.5 L × 1.75 radius cm) with air vent holes in front for animal respiration and an opening in the back for animal placement – a design similar to other restraint tubes for prairie voles (DeVries et al., 1997a). Prior to placement into these restraint tubes, immobilized females were bound (excluding the head) in plastic mesh, leading to complete physical immobilization.

Subjects exposed to social defeat experienced one of two separate paradigms to vary the intensity of the defeat. In the brief social defeat paradigm, subjects were placed into the resident cage (45 L × 22 W × 20 H cm) of an unfamiliar, unrelated, pair-bonded female prairie vole after its male partner was removed. As pair-bonded female prairie voles are territorial (Getz et al., 1981), they displayed aggressive behavior toward the intruding female subject. Subjects were exposed to physical interaction with the aggressive resident for 15 min (behavioral video confirmed this interaction). Thereafter, a plexiglass divider with air holes was placed in the center of the cage to prevent physical aggression, though nonphysical aggression was still possible, and allow the subject to create spatial distance from the aggressor, up to 22.5 cm. The social defeat exposure continued for an additional 45 min with the divider in place. In the

prolonged social defeat paradigm, the physical contact period lasted for 30 min, then the subject was placed in a small wire-mesh container (5 cm^3) in the center of the aggressor's cage for an additional 30 min. The small wire-mesh container was used to 1) prevent physical contact, 2) allow the resident to continue to display aggressive posturing and other forms of nonphysical aggression, and 3) prevent the subject from creating spatial separation between the resident and itself. Following each of the stress treatments, subjects were put into a clean, empty cage and remained alone for 30 min before they were tested on an EPM test.

Finally, a handled-control group (HAN) was created. HAN subjects were picked up and briefly handled at the same time as the four stress groups but then returned to their home cage with their partner. However, as the HAN females did not experience 30 min social isolation as the females experienced following their stress exposure, a second control group (social control, SC) was created for Experiment 1. SC females were briefly handled, stayed with their partner for 60 min, and then were transferred to a clean, empty cage for 30 min before the EPM test.

EPM Test

The EPM test was conducted for 5 min using an established method (Stowe et al., 2005). Briefly, the EPM (Columbus Instruments, Columbus, OH) is comprised of two open arms (35 L x 6.5 W cm) and two closed arms (35 L x 5 W x 15 H cm) that cross in the middle, and is elevated 45 cm off the ground. Subjects were placed in the center facing an open arm and recorded with a video/computer system. Several behaviors were quantified by a trained observer blind to the treatment using *J-Watcher V1.0* (Macquarie University and UCLA; http://www.jwatcher.ucla.edu/) for anxiety-like responses (latency to enter the open arm, percentage of time spent on the open arms vs. total arm time, and percentage of open arm entries vs. total arm entries) and locomotor activity (total arm entries).

Blood Collection and Preparation

Immediately after the EPM test, trunk blood (~400 µl) was collected following rapid decapitation into microcentrifuge vials containing 20 µl EDTA. The vials were inverted and immediately placed in ice. The entire blood collection procedure until chilling did not exceed 2 min. Blood was centrifuged at 6000 rpm for 15 min at 4 °C, then plasma was aspirated and

centrifuged at 6000 rpm for 10 min at 4 °C. Plasma was aliquoted into microcentrifuge vials and stored at -80 °C until processed via a corticosterone radioimmunoassay.

Corticosterone Radioimmunoassay

Plasma corticosterone (1:1000) was measured (in duplicates) in 10 µl plasma samples using commercially available kits (Diagnostic Products Corp., Los Angeles, CA) that have been used and validated in previous studies in prairie voles (Taymans et al., 1997; Stowe et al., 2005; Grippo et al., 2007a; Bosch et al., 2009). Other than the dilution factor, which was optimized for vole physiology, the assays were conducted according to the manufacturer's directions. The detecting limit of the radioimmunoassay kit was 7.7 ng/mL, and the intra-assay and inter-assay coefficient of variation (CV) was 2.85% and 2.11%, respectively.

Experimental Design

Experiment 1 was designed to test the influence of the source of a stressful event on the post-stress recovery of the HPA axis and anxiety-like behavior (Fig. 1). Subjects were pair-housed with an unrelated, vasectomized male for two weeks, a period that reliably leads to vole pair bonding (Aragona and Wang, 2004). Thereafter, subjects were removed from their home cage between 1100-1200 h and randomly assigned into one of the four stress paradigms, environmental novelty (n=6), immobilization (n=6), brief social defeat (n=8), or prolonged social defeat (n=7), or the control groups (HAN: n=6; SC: n=6). Following the 60 min of stress, subjects were placed in an empty, clean cage with food and water *ad libitum* for a 30-min recovery period. Thereafter, subjects were tested for their anxiety-like behaviors in the EPM test. Immediately after the EPM test, subjects were sacrificed via rapid decapitation, and trunk blood was taken and stored at -80 °C until processed via a corticosterone radioimmunoassay.

Experiment 2 was designed to evaluate the effect of previous experience with a homotypic stressor in a daily predictable (fixed) or unpredictable (irregular) schedule on the post-stress HPA axis and behavioral recovery (Fig. 2). As the immobilization stress was effective in inducing both hormonal and behavioral stress responses in Experiment 1, I focused on the immobilization paradigm. Subjects were pair-housed with an unrelated, vasectomized male for two weeks. In the predictable immobilization paradigm (pIMO), females were exposed to 60 min of immobilization (between 1100-1200 h) every day for 3 (n=8) or 7 (n=6) consecutive days

(Fig. 2A-B). In the unpredictable IMO paradigm (uIMO), the immobilization schedule was established to minimize predictability of the presentation of the stress. Several studies have utilized immobilization stress with an unpredictable schedule varying stress-rest days, immobilization duration, and time of day (Quirce et al., 1981; Rockman et al., 1987; Bryant et al., 1988; Martí and Armario, 1997). In the 3 day uIMO schedule (n=7), the schedule included immobilization on days 1 and 3 and no immobilization stress on day 2 (Fig. 2C) In the 7 day uIMO schedule (n=6), subjects were exposed to immobilization stress on days 1, 3, 4, and 7 and rested on days 2, 5, and 6 (Fig. 2D). Thirty minutes after the last immobilization, subjects were tested for their anxiety-like behaviors in the EPM test, and then sacrificed via rapid decapitation. Trunk blood was collected, stored at -80°C, and processed via a corticosterone radioimmunoassay. In addition, a HAN control group (n=11) and an acute immobilized female group (n=6) were established as in Experiment 1 to compare how pIMO and uIMO affect the biobehavioral stress response.

Data Analysis

Data were analyzed using IBM SPSS Statistics 19 (SPSS, Inc., an IBM Company) and were expressed as mean ± SEM. EPM behavior and plasma corticosterone concentrations were analyzed with a one-way ANOVA with stress condition as the single factor. Significant group differences ($p < 0.05$) were further assessed with a Gabriel's *post-hoc* test, as this test explicitly allows for unequal sample sizes. In addition, corticosterone levels were correlated to anxiety-like behavior in the EPM test for Experiments 1 and 2 by Pearson's correlations. All alpha levels were set at $p < 0.05$.

Results

Various Psychological and Social Stressors on Behavioral and Physiological Stress Response

In Experiment 1, anxiety-like behaviors in the EPM test and corticosterone levels were significantly different among groups, an effect that seemed to depend on the source and intensity of the stressor as indicated by *post-hoc* analysis. Particularly, there were group differences in the latency to enter the open arm [$F_{(5,31)} = 3.08$, $p < 0.005$] and the percentage of time spent in the

open arm [$F_{(5,33)} = 3.23$ $p < 0.05$] in the EPM test. However, *post-hoc* analyses indicated that only immobilization females delayed entrance into the open arm and decreased time spent in the open arm compared to the HAN females (Fig. 3A-B). No group differences were observed in the percentage of entries into the open arm [$F_{(5,33)} = 2.13$, $p = 0.09$] (Table 1) or in the number of total arm entries [$F_{(5,33)} = 1.77$, $p = 0.15$] (Fig. 3C), indicating that differences between immobilized and HAN females in their anxiety-like behaviors were not due to altered locomotor activity. In addition, corticosterone levels were significantly elevated following the EPM in females exposed to immobilization and prolonged social defeat stress compared to HAN females, but not females exposed to environmental novelty or brief social defeat stress [$F_{(5,33)} = 4.27$ $p < 0.005$] (Fig. 3D). It is worth noting that the effects of stress on the behavioral and physiological responses seem to be a function of the stressor rather than the brief separation from the partner during the 30 min recovery period as HAN and SC females did not differ in any of the measurements. In addition, there was a negative correlation between female corticosterone concentrations and percentage of time spent in the open arm in the EPM test [$r = -0.42$, $p = 0.01$], such that females with high corticosterone concentrations spent significantly less time in the open arm than females with low corticosterone concentrations (Fig. 3E). There was no correlation between the corticosterone concentrations and latency to enter the open arm [$r = 0.08$, $p = 0.64$] or percentage of entries into the open arm [$r = -0.13$, $p = 0.44$; data not shown].

Predictable vs. Unpredictable Immobilization on Behavioral and Physiological Stress Response

The behavioral and physiological response seemed to vary as a function of the predictability and number of exposures to repeated immobilization in female prairie voles. There were group differences in the anxiety-like behaviors in the EPM test, including latency to enter the open arm [$F_{(5,37)} = 5.14$ $p < 0.001$], percentage of entries into the open arm [$F_{(5,38)} = 3.23$ $p < 0.05$], and percentage of time spent in the open arm [$F_{(5,38)} = 5.78$ $p < 0.001$]. When *post-hoc* analyses were conducted no group differences were observed in the percentage of entries into the open arm (Table 1) and only females exposed once to immobilization significantly delayed entry into the open arm compared to HAN controls (Fig. 4A). However, females exposed to uIMO for 3 or 7 days, like females exposed to a single immobilization, significantly decreased the percentage of time spent in the open arm compared to HAN controls, while females exposed to

pIMO for 3 or 7 days were similar to HAN controls (Fig. 4B). These effects on anxiety-like behavior were not the result of altered locomotor activity as there were no group differences in total arm entries [$F_{(5,38)} = 1.19$ $p = 0.33$] (Fig. 4C).

In addition, there was a significant group difference in the plasma corticosterone concentrations [$F_{(5,38)} = 14.69$ $p < 0.001$] (Fig. 4D). Females exposed to pIMO for 3 consecutive days had corticosterone levels significantly higher than HAN controls. However, females exposed to pIMO for 7 consecutive days had corticosterone levels similar to HAN controls, indicating 7 days, rather than 3 days, of pIMO is sufficient to induce habituation. In addition, females exposed to uIMO for 3 or 7 days had corticosterone levels significantly elevated to HAN controls, like females exposed to a single day of immobilization. Furthermore, while females exposed to uIMO for 3 days had corticosterone levels similar to females exposed to immobilization for 1 day, females exposed to uIMO for 7 days had significantly higher corticosterone levels compared to females exposed to immobilization for 1 day, indicating a time course for augmentation. Females exposed to uIMO for 7 days also had significantly higher corticosterone levels than females exposed to pIMO for 7 days, indicating the predictability of immobilization affected corticosterone levels. Finally, corticosterone levels were associated with the latency to enter the open arm [$r = 0.37$, $p < 0.05$; data not shown] and percentage of time spent in the open arm [$r = -0.36$, $p < 0.05$] (Fig. 4E), but not the percentage of entries into the open arm [$r = -0.21$, $p = 0.17$; data not shown]. Females with high corticosterone levels delayed entry into the open arm more and spent less percentage of time in the open arm compared to females with low corticosterone levels.

Discussion

Stressful life events are common and can be rather disruptive to normal physiological function and behavioral routines, depending on the nature of the stressor. In the current study, the stress response by female prairie voles was dependent on stress intensity, source, and predictability as well as previous experience with the stressor. Female prairie voles exposed to immobilization (‘plastic mesh’) or prolonged social defeat displayed increased corticosterone levels after an EPM test, but only immobilized females displayed behavioral disruption (i.e., increased anxiety-like behaviors in the EPM test). This suggests that the disturbance to normal

physiological function, particularly HPA axis function, induced by immobilization is persistent and may lead to aberrant behavioral manifestations in subsequent stressful conditions. Thus, I further evaluated the effects of repeated immobilization, modulating predictability of the exposure. Females exposed to pIMO displayed physiological (7 days repeated) and behavioral (3 and 7 days repeated) habituation, while females exposed to uIMO did not display habituation. In fact, females exposed to uIMO for 7 days had augmented corticosterone levels. It seems that the behavioral and physiological response to the EPM test following repeated immobilization depended on the predictability of the stress as well as the number of days of repeated immobilization. Furthermore, the HPA axis response, as evident by corticosterone levels, was associated with the impact that these factors had on behavioral routines.

The stress response can be non-specific, the general adaptation syndrome described by Hans Selye (1936), but there are components of the stress response that are adaptive to specific environmental cues, creating a stressor-specific response (reviewed in Armario, 2006). Regarding the HPA axis, circulating corticosterone concentrations during stress are related to the intensity of stress in response to low to intermediate intensity stressors, but not high intensity stressors (Hennessy and Levine, 1978; Hennessy et al., 1979; Natelson et al., 1981; Armario et al., 1986a; Armario et al., 1986b). This is partially due to the fact that maximal adrenal steroidogenesis can be produced from an intermediate ACTH release, disassociating ACTH and corticosterone release during high intensity stressors (Keller-Wood et al., 1981). However, when post-stress corticosterone levels are considered, high intensity stressors provide distinctive recovery rates (García et al., 2000; Marquez et al., 2002). In the current study, corticosterone levels were significantly elevated following an EPM test in female prairie voles recovering from immobilization but not environmental novelty. Furthermore, corticosterone levels were elevated following the EPM test in females exposed to prolonged social defeat paradigm, which included longer physical confrontation and lack of control/no escape during non-physical confrontation, but not in females exposed to the brief social defeat paradigm. Therefore, the intensity of social defeat may vary as a function of duration of the physical confrontation or control during the confrontation—control referring to the capability of the vole to avoid the aggressive conspecific by making an appropriate motor response (i.e., moving to the maximal distance away from the aggressive conspecific), a definition adapted from Levine (1985). Together, these data suggest that environmental novelty induces mild psychological stress while immobilization and social

defeat reflects more severe stress in prairie voles, similar to other rodent species (Korte and De Boer, 2003; Armario, 2006). In addition, these data demonstrate that prairie voles may be utilized as an ethologically-relevant rodent model of female social defeat. This is valuable as animal models of female social defeat are lacking as females from traditional laboratory rodents do not reliably fight each other in a resident-intruder confrontation, with the exception of maternal aggression (Björkqvist, 2001; Bosch et al., 2004; Huhman, 2006).

Furthermore, I evaluated the behavioral response of stressed females to a secondary stressor, the EPM test. While immobilization and prolonged social defeat led to a rise in plasma corticosterone levels following an EPM test, only immobilization induced anxiety-like behavior on the EPM test. A simple interpretation would be that immobilization is a more intense stressor than social defeat and therefore led to the behavioral manifestations. However, it would be worth noting that the EPM test evaluates a non-social anxiety-like response, and therefore, social stress, like social defeat, may not induce anxiety-like behavior in this context. For example, Barsy and colleagues (2010) noted that restraint stress evokes a generalized anxiety response in rats. Specifically, restrained rats displayed social and non-social anxiety-like behaviors indexed by social avoidance in a social interaction test (SIT) and avoidance of the open arms in an EPM test, respectively. By comparison, social defeat induced social avoidance in the SIT in rats but had no effect on EPM behavior. Thus, social stress may selectively induce social anxiety, or anxiety-like behavior within a social context, while psychological stress induces a general anxiety-like state. In fact, several studies have observed that different types of stress can lead to different behavioral consequences (e.g., psychological vs. social stress: van Erp et al., 1994; Doremus-Fitzwater et al., 2009; psychological vs. physical stress: Daviu et al., 2012; physical vs. social stress: McBlane and Handley, 1994; Gasparotto et al., 2005; environmental vs. psychological stress: Muñoz-Abellán et al., 2008; Munoz-Abellan et al., 2011).

For several decades, it has been known that the magnitude of the HPA axis response to a stressor, even the more intense stressors like immobilization, declines with repeated exposures. For example, repeated restraint or immobilization stress can facilitate a reduction in the secretion of peripheral HPA axis hormones (i.e., ACTH and corticosterone) and depress the stress-induced neuronal activation (e.g., *c-fos* and CRH gene expression) in the PVN and other brain regions that regulate the stress-induced CRH action in the PVN (e.g., the hippocampus and amygdala) (Melia et al., 1994; García et al., 2000; Pinnock and Herbert, 2001; Armario et al., 2004). This

depressed response toward a homotypic stressor has been referred to as habituation (Thompson and Spencer, 1966). As predicted, female prairie voles exposed to repeated pIMO (three sessions: 3 day pIMO; seven sessions: 7 day pIMO) displayed a habituated behavioral and physiological response. However, voles exposed to uIMO (two sessions: 3 day uIMO; four sessions: 7 day uIMO) displayed an augmented corticosterone response and lack of behavioral habituation. Under the pIMO and uIMO paradigms, female prairie voles were exposed to the same intense immobilization stress for the same duration (1 h). Thus, the difference in the adaptation to pIMO compared to uIMO seems to be independent of these characteristics. While increasing the number of exposures may have influenced the habituation of females exposed to pIMO, it did not facilitate habituation in females exposed to uIMO. Moreover, females exposed to three immobilization sessions during the 3 day pIMO schedule displayed a reduced anxiety-like behavioral response, while the females exposed to four immobilization sessions during the 7 day uIMO schedule did not habituate behaviorally, and even displayed an augmented corticosterone response. It seems likely that the longer and more irregular intervals between immobilization exposures observed in uIMO compared to the shorter and consistent intervals in the pIMO schedule facilitate the differences in adaptations. In order to further understand the influence that stress predictability has on the underlying neuroendocrine mechanism that governs stress habituation or augmentation in voles, it will be worth evaluating the influence of repeated pIMO and uIMO on various markers of stress-induced neuronal activation, including *c-fos* and CRH gene expression in the PVN and other brain regions that regulate the stress-induced CRH action in the PVN. As well-documented in other rodent species (Armario et al., 1988; Martí and Armario, 1998; Grissom and Bhatnagar, 2009), repeated stress may have influenced adrenal sensitivity in female prairie voles; thus, additional research needs to be conducted to determine the influence of pIMO and uIMO on the ACTH response and changes to adrenal weight.

Prairie voles are not commonly used in stress research. Nonetheless, features of vole physiology and their social system make them a promising rodent model of stress. The fact that I measured plasma corticosterone concentrations 30 min after a primary stressor and immediately following a secondary stressor (EPM test) makes interpretation of the dynamics of the corticosterone response more challenging. Nonetheless, these current results suggest that vole physiology is adaptive to the stress intensity, source, and predictability as well as previous experience with a stressor. Prairie voles exhibit high basal plasma corticosterone levels, 5 to 10

times higher than rats and mice (Taymans et al., 1997). However, voles do not display common consequences associated with chronic hypercortisolism, potentially due to a suppressed corticosterone signal (Taymans et al., 1997; Hastings et al., 1999). Therefore, prairie voles could provide a valuable rodent model of glucocorticoid resistance, and understanding which characteristics of a stressor modulate this response is necessary for such research. Furthermore, the prairie vole social system has many similarities to human society that are not reflected in the social systems of more traditional laboratory rodents, including male-female pair-bonds, male and female territoriality, biparental care, and extended families (Getz et al., 1981; Carter et al., 1995a; Keverne and Curley, 2004). Moreover, the social environment has a major influence on the stress system. Therefore, as more emphasis is given to understand how different facets of the vole social system influences the stress response, knowledge about how these same factors regulate human stress may be gleaned.

CHAPTER THREE

HYPOTHALAMIC OXYTOCIN MEDIATES SOCIAL BUFFERING OF THE STRESS RESPONSE

Adapted from:

Smith, A.S. and Wang, Z. (in review). Hypothalamic oxytocin mediates social buffering of the stress response. *Biol Psychiatry*.

Introduction

Stressful life events (e.g., divorce or death of a spouse) are deleterious to adult mental health in humans (Brown, 1989; Dalgard et al., 2006), and the social environment can either propagate or attenuate these effects. For example, depression is concomitant with the lack of social support following a stressful life event (Dalgard et al., 2006). In contrast, close relationships can ameliorate stress-induced biobehavioral responses in humans and, therefore, reduce the risk of psychological disorders (Cohen and Wills, 1985; Paykel, 1994; Smith and Wang, 2012). For instance, the natural occurrence of physical touch with an infant during pre-feeding behaviors and nursing is sufficient to reduce the anxiety experienced by mothers during physical and psychological stress (Carter and Altemus, 1997). This social buffering effect has also been observed through contact with a committed social partner, reducing the negative impact of stress (e.g., suffering from a panic disorder or psychological distress; Cohen and Wills, 1985; Paykel, 1994; Smith and Wang, 2012). Still, the neuroendocrine mechanism underlying social buffering via committed partnerships is not well understood, and there are limited modeling of this phenomenon in animal research as less than 3% of mammalian species are monogamous and display social bonding between partners.

The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent that forms long-term adult bonds, called pair bonds (Aragona and Wang, 2004). These bonds may protect against the aversive effects of stress by attenuating the action of the hypothalamic-pituitary-adrenal (HPA) axis (DeVries et al., 2007). For example, while prolonged social separation elevates basal levels of corticosterone and increases depression-like response to acute psychological stressors (Bosch et al., 2009), social pairing can reduce basal corticosterone levels

(DeVries et al., 1995; DeVries et al., 1997b). Further, reunion with a social partner can attenuate the HPA axis response associated with separation (Carter et al., 1995b). Several neuroendocrine systems including oxytocin (OT), vasopressin (AVP), and corticotrophin-releasing hormone (CRH) are involved in regulating prairie vole pair bonds (Aragona and Wang, 2004; Hammack et al., 2012). Interestingly, these systems are vital to the regulation of the stress-induced HPA axis activation. In response to stress, the paraventricular nucleus (PVN) of the hypothalamus releases CRH and AVP to promote a signaling cascade, leading to an increase in circulating corticosterone and an induction in psychological and behavioral pathologies (Smith and Wang, 2012). In contrast, OT released from the PVN in rats acts as an anxiolytic—suppressing HPA axis function (Neumann et al., 2000b). Therefore, the neuroendocrine systems that are impetuses to adult social bonds may also regulate the social buffering of the stress response.

I set out to investigate the effects of social support on the behavioral and hormonal response to psychological stress as well as the underlying neuroendocrine mechanisms of social buffering in female prairie voles. First, I established a behavioral paradigm demonstrating that social support from a male partner following immobilization stress decreases the behavioral and hormonal stress response and increases dyadic interaction, and that social-promoting neuroendocrine systems may be involved. Specifically, I observed that social recovering following stress alters the action of the PVN OT system while suppressing the stress response. Therefore, in the subsequent experiments, I focused on the functional role of the OT system in regulating the anxiolytic effects of social buffering. These data demonstrate that social recovery following a stressful event can facilitate OT release in the PVN to promote social buffering.

Methods

Subjects

Subjects were captive-bred female prairie voles (*M. ochrogaster*) descended from populations in southern Illinois. Voles were weaned on postnatal day 21 and housed with a same-sex conspecific in plexiglass cages (29 L × 18 W × 13 H cm) containing cedar chip bedding with food and water *ad libitum*. Colony rooms were maintained on a 14L:10D photoperiod (lights on at 0700 h) and at a temperature range of 21±1 °C. All female subjects that were used in this study were sexually naïve, prior to pairing with a male cagemate. Female prairie voles were pair-

housed with an unrelated, vasectomized male for two weeks, a sufficient time period that reliably leads to pair-bond formation in prairie voles (Aragona and Wang, 2004). Experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Florida State University.

Immobilization Stress

Physical restraint has been used in a number of different rodent animal models to induce stress, including prairie voles (Smith et al., in press). On the day of testing, females were exposed to an acute immobilization stress paradigm that the Wang lab has recently developed to elicit a behavioral and physiological stress response in female prairie voles. Subjects were exposed to 1 h immobilization stress in restraint tubes constructed from 50 mL centrifuge tubes (115 L x 29 OD mm) with the top third of the tube removed to allow for adjusting the diameter for the size of the animal and unrestricted respiration. Prior to placement into these restraint tubes, the fore- and hind-limbs of immobilized females were bound with double-sized Velcro straps. The animals were placed into the restraint tubes, then a second set of straps was placed around the outside of the tube that covered over the body and head of the animal, leading to complete physical immobilization.

Behavioral Measures

The EPM test was conducted for 5 min using an established method (Smith et al., in press). Briefly, subjects were placed in the center facing an open arm and recorded with a video/computer system. Several behaviors were quantified by a trained observer blind to the treatment using *J-Watcher V1.0* (Macquarie University and UCLA; http://www.jwatcher.ucla.edu/) for anxiety-like responses (latency to enter the open arm, percentage of time spent on the open arms vs. total arm time, and percentage of open arm entries vs. total arm entries) and locomotor activity (total arm entries). In addition, the occurrence and duration of female stress-related behaviors and female and male pro-social behaviors were recorded for 1 h during baseline conditions while in the home cage and 1 h during the recovery period after the female was immobilized. Stress-related behaviors included rearing, autogrooming, and route tracing, while pro-social behaviors included olfactory investigating, initiating contact with/approaching, following, and allogrooming the partner. Male sexual

behavior (i.e., mounting) was also recorded. These behaviors were quantified by a trained observer blind to the treatment using *J-Watcher V1.0* (Macquarie University and UCLA; http://www.jwatcher.ucla.edu/). The occurrence of these behaviors were divided into 30 min periods, and a raw change score was calculated for each behavior (post-stress values minus pre-stress values).

Blood Preparation and Corticosterone Radioimmunoassay

Trunk blood (~400 µl) was collected following rapid decapitation into microcentrifuge vials containing 20 µl EDTA. The vials were inverted and immediately placed in ice. The entire blood collection procedure until chilling did not exceed 2 min. Blood was centrifuged at 6000 rpm for 15 min at 4 °C, then plasma was aspirated and centrifuged at 6000 rpm for 10 min at 4 °C. Plasma corticosterone (1:1000) was measured (in duplicates) in 10 µl plasma samples, using a commercially available kit (Diagnostic Products Corp., Los Angeles, CA) previously used and validated in prairie voles (Bosch et al., 2009; Smith et al., in press). The detecting limits of the radioimmunoassay kit was 7.7 ng/mL for corticosterone. The intra-assay coefficient of variation (CV) was 2.85%, and all samples were measured in a single assay.

Brain Tissue Preparation and Protein Extraction

After rapid decapitation, brains were collected and then frozen at -80 °C. Brains were sectioned coronally at 300 µm and thaw-mounted onto Superfrost/plus slides. Based on the involvement in the stress response, social interactions, and/or presence of OT, AVP, or CRH system, a number of brain regions were selected for analysis including the PVN, NAcc, CeA, and medial amygdala (MeA) (Gimpl and Fahrenholz, 2001; Aragona and Wang, 2004; Engelmann et al., 2004; Smith and Wang, 2012). These brain regions were identified using the Paxinos and Watson rat brain atlas (PVN: Plates 42–49, NAcc: Plates 9–11, MeA: Plates 48–63, and CeA: Plates 48–58; Paxinos and Watson, 1998). Bilateral tissue punches of a 1 mm diameter were taken from three sections of each brain region and stored at −80 °C until processed. Brain tissue punches were lysed through sonication and centrifuged in a RIPA buffer. The total protein concentration of each sample (5 µl) was assayed using a DC protein assay (BioRad Labs; Hercules, CA) and read by Gen 5.1 and a Biotek microplate reader.

Western Blots for Vasopressin, CRH, Oxytocin Receptor, and V1a Receptor

Protein extract from the brain tissue samples (20 µg of total protein) was electrophoresed on precast 10–20% SDS gradient gels. After transferring the protein from the gel to a PVDF membrane, the membrane was washed using 1X Tris-buffered saline with 0.1% Tween-20 (TBS-T) and then blocked in 5% w/v milk for 1 h at room temperature (~23 °C). After the general preparation of the membrane, the membrane was incubated with an antibody appropriate for vasopressin, CRH, oxytocin receptor, or V1a receptor diluted in a blocking buffer at 4 °C for various lengths of time (Table 2). Subsequently, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody that labels the primary antibody at 25 °C for 1 h, and bands were visualized by enhanced chemiluminescence on x-ray film. After the visualization step for each marker, the membrane was incubated in a stripping solution and then blocked in 5% w/v milk for 1 h at room temperature before repeating the process for the next marker. The optical densities for vasopressin, CRH, oxytocin receptor, and V1a receptor labeling on x-ray film for each sample were quantified using a computerized image program (Image J, NIH). These values were normalized for band intensities using the optical density of β-actin for that sample and standardized by the average normalized optical density of that marker for the HAN controls.

Oxytocin Enzyme Immunoassay

OT concentrations were measured (in duplicate) in the tissue punch protein extracts from the PVN, supraoptic nucleus of the hypothalamus (SON), NAcc, CeA, and MeA using an enzyme immunoassay kit (Assay Designs INC., Ann Arbor, MI, USA). Non-extracted samples were diluted at 1:20 for OT (5 µl of protein extract) and assayed according to kit instructions. The total OT content for each sample was corrected by the total protein content of that sample. The detecting limit of the enzyme immunoassay kit was 11.7 pg/mL. The intra-assay and inter-assay CV was 2.56% and 3.70%, respectively. In addition, OT concentrations were determined from the dialysate samples using the sample protocol. The intra-assay and inter-assay CV was 1.32% and 4.17%, respectively. The OT concentration was not detectable in the NAcc, CeA, or MeA.

Intra-PVN Administration of Oxytocin and Oxytocin Receptor Antagonist

After 7 d of pairing with a male cagemate, female voles were anesthetized and then stereotaxically implanted with 26-gauge guide cannulae (Plastics One) aimed at the PVN (nose bar; AP, -0.74 mm; ML, ± 1.5 mm; DV, 5.0 mm; angle, ± 15°). The voles were allowed 7 d of post-operative recovery. On the day of testing, voles were exposed to the 60-min immobilization stressor. Following this stressor, OT (10 ng or 100 ng), or a selective oxytocin receptor antagonist (10 ng or 100 ng; des-Gly-NH2,d(CH2)5(Tyr(Me)2,Thr4)OVT, kindly provided by Dr. Maurice Manning, Toledo, OH, USA) was dissolved in artificial cerebrospinal fluid (aCSF) and delivered to immobilizated voles by infusion in a 200 nl volume over 90 s. Vehicle-treated voles were injected with 200 nl aCSF. At the end of the experiment, brains were collected for histological verification of probe placement.

PVN Microdialysis

Microdialysis probe construction, cannulation, and dialysate collection were previously described (Curtis et al., 2003). Briefly, the active area of the dialysis membrane was 1.0 mm and had a molecular weight cutoff of 18kDa. Probes were perfused continuously at 1.0 L/min with an aCSF solution using a glass Hamilton syringe connected to an automatic micropump (World Precision Instruments). Voles were anesthetized and then stereotaxically implanted with a microdialysis probe aimed at the PVN (nose bar; AP, -0.7 mm; ML, 0.2 mm; DV, 7.15 mm). Voles were given 24 h to recover, then dialysate samples were collected every 15 min for eight consecutive samples before immobilization stress (only samples 5-8 were used as baseline as the first four were collected to acclimate the vole to the collection process), during the 1 h immobilization stress, and during the 1 h post-immobilization recovery period into vials containing 5µl 0.1 N HCl. Samples were immediately frozen on dry ice. The final four baseline samples were pooled together, as OT concentrations were too low for detection in these samples during pilot experiments. In addition, the first and last two samples during the stress period and the recovery period were pooled. After collection was complete, all samples were stored at -80°C until processed via the OT enzyme immunoassay.

Data Analysis

ANOVAs were used for experiments that compared effects of 1) recovery conditions, 2) recovery conditions and time, and 3) recovery conditions and drug treatment, and the Student-Newman-Keuls (SNK) *post-hoc* test was used if any main effects or interactions reached statistical significance [$p < 0.05$]. One-sample and paired *t-tests* were used to evaluate the changes in stress-related and pro-social behaviors (raw change scores were calculated for each individual by subtracting pre-stress values from post-stress values), respectively. Data were analyzed using IBM SPSS Statistics 19 (SPSS, Inc., an IBM Company) and were expressed as mean ± SEM.

Results

Social Support Attenuated Behavioral and Hormonal Responses to Immobilization Stress

I first characterized the impact of recovering with a male partner following psychological stress on the behavioral and hormonal response in female prairie voles. Pair-bonded female voles received 1 h immobilization stress, recovered either alone or with their pair-bonded male partner for 30 min, and then were examined for their anxiety-like behaviors in an elevated plus maze (EPM) test and circulating levels of corticosterone. Immobilized females recovering alone displayed a substantial increase in EPM anxiety-like behaviors, including delayed open arm latency [$F_{(3, 17)} = 4.93$, $p < 0.05$], fewer open arms entries [$F_{(3, 22)} = 3.86$, $p < 0.05$], and reduced open arm duration [$F_{(3, 22)} = 5.14$, $p < 0.01$], compared to the handled controls (Fig. 5A-B), similar to previous reports (Smith et al., in press). The former also had a rise in circulating corticosterone levels compared to the latter [$F_{(3, 21)} = 6.32$, $p < 0.01$] (Fig. 5D). By contrast, recovering with a male partner (social support) following immobilization stress attenuated anxiety-like behaviors and blunted the rise in circulating levels of corticosterone, mirroring responses in the handled controls. This effect seems to be behavior-specific as the number of total arm entries, a locomotor measure, was similar for all groups (Fig. 5C). Furthermore, as prairie voles are sensitive to social separation or isolation, which may affect HPA axis function and performance on the EPM (Grippo et al., 2008; Bosch et al., 2009; Lieberwirth et al., 2012), I included a cohort of non-immobilized females that were removed from their male partner (i.e., social control) during the 30 min recovery period designed for other groups. These females did

not differ from the handled controls in anxiety-like behaviors or circulating levels of corticosterone (Fig. 5). Together, these data demonstrate that the immobilization-induced stress response can be buffered by social support from a bonded partner.

Social Support Suppressed Female Stress-related Behaviors and Promoted Dyadic Interaction

I also evaluated the female prairie vole behavioral stress response by comparing the occurrence of stress-related behaviors (i.e., rearing, repetitive autogrooming, and route tracing) for 1 h while females remained undisturbed in their home enclosures with their male partner and again for 1 h post-immobilization while they recovered alone or with their male partner. I observed immobilization-induced changes in stress-related behaviors in the recovery chamber compared to baseline conditions that were dependent on the social environment [stress index frequency, $t_{(11)} = 4.00$, $p < 0.005$; stress index duration, $t_{(11)} = 3.83$, $p < 0.005$] (Fig. 6A-B). Specifically, females that recovered alone displayed a significant increase in these stress-related behaviors, namely route tracing [frequency, $t_{(5)} = 3.07$, $p < 0.05$; duration, $t_{(5)} = 2.70$, $p < 0.05$] and repetitive autogrooming [frequency, $t_{(5)} = 4.37$, $p < 0.01$; duration, $t_{(5)} = 4.82$, $p < 0.005$]. These effects were noted during the first 30 min following exposure to immobilization, returning to baseline levels 30 min later (Table 3). However, among immobilized females recovering with their male partner, I observed no changes in stress-related behaviors initially (Fig. 6A-B). After interacting with their male partner for 30 min, immobilized females displayed a significant drop in autogrooming [frequency, $t_{(6)} = -2.73$, $p < 0.05$; duration, $t_{(6)} = -3.06$, $p < 0.05$] (Table 3). The occurrence of and time spent route tracing were not different at this latter recovery period compared to baseline (Table 3). However, this null effect may be due to the fact that seven of the eight females recovering with their male partner never displayed this behavior during the baseline condition (inducing a flooring effect). These data suggest that the occurrence of stress-related behaviors was augmented when the female prairie voles recovered alone but was suppressed when recovering with a social partner.

In humans and other gregarious mammals, stress can stimulate social seeking behaviors (Simsek et al., 2012), and increased pro-social behaviors can lead to a reduction in the stress response (Smith and Wang, 2012). I did not observe a substantive change in female pro-social behaviors after immobilization (Fig. 6C-D and Table 3). However, male prairie voles augmented

their pro-social behaviors during the first 30 min when their female partner returned from exposure to immobilization stress—approaching [frequency, $t_{(6)} = 2.91$, $p < 0.05$], sniffing [frequency, $t_{(6)} = 2.74$, $p < 0.05$], and grooming [frequency, $t_{(6)} = 3.80$, $p < 0.01$; duration, $t_{(6)} = 3.31$, $p < 0.05$] the female more often (Fig. 6E-F). Such effects were not observed in the subsequent 30 min (Table 3). In addition, mating behavior was limited and did not differ between pre- and post-stress periods; notably, only one of the eight pairs were observed mating during the pre- and post-stress periods. Therefore, as females recover from immobilization with their male partner, they experience an enhanced pro-social display from their male partner and concomitantly display less stress-related behaviors.

Immobilization Affected Neuropeptide Receptor Expression in a Brain Region-specific Manner

Western blotting revealed that the 1 h immobilization stress reduced the expression of OT receptors (OTR) [$F_{(3, 19)} = 5.37$, $p < 0.01$] (Fig. 7A) and AVP V1a receptors (V1aR) [$F_{(3, 9)} = 5.88$, $p < 0.05$] (Fig 3.3B) in the PVN, regardless of whether the female voles recovered alone or with a male partner. Such effects on OTR and V1aR were not found in other brain areas, including the nucleus accumbens (NAcc) and the medial (MeA) and central (CeA) nuclei of the amygdala, indicating that the effect was brain region-specific (Table 4). Data from other rodent species have shown that stress can increase OT and AVP release in the PVN (Engelmann et al., 2004), and persistent OT and AVP release can lead to receptor desensitization and internalization within 30 min (Evans et al., 1997; Fukunaga et al., 2006). Subsequently, internalized OTRs undergo endocytosis and some V1aRs are not recycled back to the cell surface (Gimpl and Fahrenholz, 2001). Therefore, decreased OTR and V1aR expression in the PVN may indicate immobilization-induced activation of both systems.

Oxytocin was Released in the PVN During Immobilization and Social Buffering

I measured the total content of OT via enzyme-immunoassay (ELISA) as well as AVP and CRH via Western blotting in the PVN (Fig 3.3C-E), NAcc, MeA, and CeA (Table 4). As OT content was not detectable in the NAcc or amygdalar nuclei, I also measured OT in the supraoptic nucleus (SON) of the hypothalamus (Table 4). Immobilized females that recovered with a male partner had a significantly lower level of OT in the PVN compared to other groups

[$F_{(3, 22)} = 4.47$, $p < 0.05$] (Fig. 7C). No group differences were found in the OT, AVP, or CRH content in any of the other brain regions measured (Fig 3.3C-E, Table 4). Therefore, the social buffering-induced decrease in the PVN OT was brain region- and peptide-specific.

A decrease in the PVN OT content may be due to changes in OT synthesis, release, or both. In female rats, OT mRNA expression in the PVN is significantly increased 30 min following immobilization stress (Jezova et al., 1995), and social contact facilitates OT activity (Neumann, 2008). Therefore, a decrease in PVN OT content following immobilization and subsequent social buffering may be more likely due to an increased OT release. I used brain microdialysis with ELISA to measure extracellular OT concentrations in the PVN in immobilized female voles that recovered alone or with their male partner (Fig. 8A). As compared to baseline sample concentrations, immobilization significantly increased OT concentrations in the PVN in all females [$F_{(4, 44)} = 24.76$, $p < 0.0001$] (Fig. 8B), similar to reports in rats (Jezová et al., 1993; Babygirija et al., 2012). When immobilized females recovered alone, PVN OT concentrations returned to baseline levels, demonstrating no lasting release associated with the stress. Intriguingly, when females recovered with their male partner, PVN OT concentrations were substantially increased above baseline levels as well as concentrations during recovery in females recovering alone [$F_{(4, 44)} = 4.48$, $p < 0.005$] (Fig 3.4B). Therefore, recovering with a male partner following immobilization stress reduced OT content by promoting its release in the PVN.

PVN Oxytocin Mediated the Social Buffering Effect

Social buffering is an effective anxiolytic in humans before a stressor (Heinrichs et al., 2003) or afterward (Simsek et al., 2012). In rodents, intra-PVN microinjections of OT can reduce stress-induced rises of circulating corticosterone and anxiety-like behavior to acute psychological stressors (Smith and Wang, 2012). As these data demonstrate that social recovery with a male partner alleviates the behavioral and hormonal stress response and promotes the local release of OT in the PVN, I hypothesized that PVN OT mediates the social buffering effect on the stress response in female prairie voles.

Female prairie voles received bilateral stereotaxic implantations of cannulae aimed to the PVN (Fig. 9A). After 1 h immobilization stress, they were randomly assigned into one of the two groups that recovered alone or with their male partner. Immediately prior to the recovery period,

females recovering alone received bilateral intra-PVN microinjections of a vehicle (artificial cerebrospinal fluid or aCSF; 200nl/side) or vehicle containing a low (10ng/200nl/side) or a high (100ng/200nl/side) dose of OT. Females recovering with their male partner received bilateral intra-PVN microinjections of aCSF or aCSF containing a low (10ng/200nl/side) or a high (100ng/200nl/side) dose of a selective OTR antagonist.

As shown previously (Fig. 5), immobilized voles recovering with a male partner displayed significantly lower levels of anxiety-like behaviors, including delayed open arm latency [$F_{(5, 40)} = 5.12$, $p < 0.001$], fewer open arms entries [$F_{(5, 40)} = 12.78$, $p < 0.0001$] and reduced open arm duration [$F_{(5, 40)} = 8.32$, $p < 0.0001$], as well as a reduced level of circulating corticosterone [$F_{(5, 40)} = 5.27$, $p < 0.001$], compared to the voles recovering alone (Fig 3.5B-F). Microinjections of OT in the PVN decreased anxiety-like behaviors and reduced circulating levels of corticosterone in the female voles recovering alone, indicating that OT is an anxiolytic. In contrast, intra-PVN injections of OTR antagonist increased anxiety-like behaviors and elevated circulating levels of corticosterone in the female voles recovering with a male partner. Together, these data suggest that PVN OT is both necessary and sufficient for mediating social buffering effects on biobehavioral responses to stress in female prairie voles.

Discussion

Previous research has demonstrated the beneficial impact of social ties in adulthood on the physical, psychological, emotional, and behavioral responses toward a stressful event (Cohen and Wills, 1985; Paykel, 1994; Smith and Wang, 2012). The current study provides evidence for a neural mechanism underlying the social buffering effect from a pair bonded partner in female prairie voles. Female voles displayed buffered anxiety-like behavior and levels of stress hormones when recovering from psychological stress with their bonded male partner. Furthermore, OT was released in the PVN during the recovering with a social partner, and released OT played a functional role in mediating the social buffering effects on biobehavioral responses to stress. Together, these data demonstrate that PVN OT is one mechanism through which social support buffers the stress response in female prairie voles.

Social Buffering: An Impetus for Social Living

The driving forces that facilitate social attachments, including pair bonding, in numerous species include (1) reinforcing social behaviors through activating the brain reward centers, (2) the consequences of disruption or separation of close social bonds on an individual's well-being and emotional state, and (3) the beneficial effects of close social contact to improve mental and physical health (Neumann, 2009). Previous research has already documented that pair bonding in prairie voles are facilitated by the brain reward circuitry reinforcing bond-related behaviors and the consequences associated with bond absence or loss. For example, dopamine transmission within the NAcc promotes partner preference formation and maintenance of pair bonds in prairie voles (Aragona and Wang, 2004). In addition, the absence of social contact in prairie voles can promote a disruption to normal HPA axis activity and behavioral routines that mimic symptomatology of depression and anxiety disorders in humans (Grippo et al., 2008; Lieberwirth et al., 2012). Moreover, prolonged social separation from a bonded partner can elevate basal levels of corticosterone in circulation and CRH mRNA expression in the brain as well as increase a depression-like response to acute psychological stressors in prairie voles (Bosch et al., 2009). The current study focuses on the third force of social attachment, utilized the strong bond between adult male-female pairs in prairie voles to characterize the beneficial effects of social interactions with a partner following a stressful event on the recovery of anxiety-like behavior, the HPA axis, and other neuroendocrine systems that may modulate the stress response. These data demonstrate that social support attenuates the stress response as the immobilization-induced increase in stress-related behaviors and circulating corticosterone levels were eliminated when females recovered with their male partner. This extends results from previous research that has indicated social housing with a male, but not female, can reduce the baseline HPA axis activity in female prairie voles (DeVries et al., 1995). Therefore, interactions with a social partner seem to function as an anxiolytic agent in female prairie voles who live in a fundamentally social environment and benefit their emotional state and well-being.

Further, researchers have found that experience of a stressful event can attract group members and social partners together (Simsek et al., 2012), and this can lead to a reduction in their stress levels (Smith and Wang, 2012). For example, rats and humans are more likely to affiliate when under distress or following a stressful event. Thus, one potent stress buffering

strategy is to seek or utilize support provided by a social partner or social network. While I did not observe a substantive change in social seeking behavior in female prairie voles after experiencing psychological stress, their male partners augmented their pro-social behavior. Particularly, after olfactory investigation, a common response to novel or returning familiar conspecifics in the environment in rodents, males established contact with the female and engaged in social grooming. From rats to humans, individuals will act to attenuate distress in group members and significant others. For example, rats will free cagemates from restraints, acting deliberately to extinguish distress in another (Ben-Ami Bartal et al., 2011). Furthermore, consoling distressed individuals following social conflicts seems to be a critical behavior for stress coping in greater apes (Fraser et al., 2008). In addition, women who receive more hugs and other forms of "warm" physical contact from a committed partner (e.g., cohabiting partner or husband) can experience a suppression in the stress response (Dunbar, 2010). Thus, like humans and other highly social mammals, female prairie voles may experience social buffering effects through received or enacted support from a primary group member, in this case their pair-bond partner.

Efficacy of OT to Promote the Social Buffering Effect

While a number of biological pathways undoubtedly contribute to the social buffering of the stress response, the convergence of evidence denotes a role for OT in regulating social bond-promoting behaviors and their buffering effects on the stress response. The beneficial effects of social interaction on stress reduction seems to be associated with a release of OT, and disruption of OT action can inhibit the social buffering effect. For example, positive communication and "warm" physical contact between married couples are associated with higher plasma OT levels (Gordon et al., 2011), which, in turn, are correlated with decreased levels of blood pressure and cardiovascular activity (Dunbar, 2010). In male rats, mating releases OT in the PVN and facilitates a reduction in anxiety-like behavior during psychological stress (Waldherr and Neumann, 2007). These data depict a similar story as social support promotes the release of OT in the PVN while reducing the stress response in female prairie voles. However, unlike OT release in the PVN in male rats (Waldherr and Neumann, 2007), the OT release in the PVN in female prairie voles seems to be independent of sexual interaction. Of the eight females recovering from immobilization stress with their male partner, only one was observed mating

with the male partner during the recovery period, yet OT release in the PVN was significantly elevated in all eight females. Sexual encounters have been demonstrated to be sufficient stimuli to evoke OT release in female prairie voles (e.g., NAcc: Ross et al., 2009); however, the lack of mating in the current study suggests that another aspect of the social interaction with their male partner evoked the OT release in the PVN. The natural occurrence of skin-to-skin contact, a source of cutaneous warmth and tactile stimulation, that occurs between the mother and infant during infant pre-feeding behaviors and nursing is sufficient to evoke OT release in newborn rat pups (Kojima et al., 2012) and postpartum female rats (Bosch and Neumann, 2012), which is thought to originate from centrally projecting PVN neurons (Pedersen and Boccia, 2002). In this study, male partners established side-by-side contact with and groomed the female voles more often after the female returned from the immobilization stress. This increased social contact may have provided the chemosensory or somatosensory cues necessary to evoke an increased release of OT in the PVN in female prairie voles. In addition, the social buffering effect seems to rely on OT action as pharmacological blockade of OTR in the brain, via intracerebroventricular injections of an OTR antagonist, can disrupt alleviation of depression-like and anxiety-like behaviors induced by social housing in mice and mating in rats (Waldherr and Neumann, 2007; Norman et al., 2010). These data also demonstrate that despite recovering from psychological stress with a social partner, female prairie voles do not display stress relief if concomitantly receiving an injection of a selective OTR antagonist. Further, this is the first evidence to demonstrate that the social buffering effect by a committed social partner requires the activation of OTR in the PVN, providing site-specificity to the model. These data coincide with other reports that inhibition of OT action in brain regions that release OT during psychological stress (e.g., PVN: Bosch et al., 2004 and CeA: Ebner et al., 2005) can attenuate the stress-induced physiological and behavioral response (Neumann et al., 2000a; Ebner et al., 2004). Thus, these findings support the notion that OT is one mechanism through which social buffering protects from the negative impact of stressful life events.

Analogous to social support, OT appears to function as an anxiolytic agent. Social isolation activates the HPA axis and promotes depression- and anxiety-like behaviors in socially monogamous (e.g., hamsters and prairie voles) and gregarious (e.g., rats and mice) rodents (Detillion et al., 2004; Waldherr and Neumann, 2007; Grippo et al., 2008; Norman et al., 2010; Lieberwirth et al., 2012). By contrast, OT administered during periods of social isolation can

inhibit many of these negative effects (Detillion et al., 2004; Grippo et al., 2008; Norman et al., 2010; Lieberwirth et al., 2012). For example, female prairie voles display more depression-like behavior in response to psychological stress and symptoms of anhedonia (e.g., a diminished sucrose preference) when living in social isolation; yet, subcutaneous administration of OT during social isolation can eliminate these depression-like symptoms (Grippo et al., 2008). These data demonstrate that when a social partner is unavailable after a stressful event, the female prairie vole stress response can be alleviated via an intra-PVN OT injection. Therefore, the negative impact of stress can be attenuated by OT treatments, even in the absence of social contact.

Finally, there is a variety of anatomical and functional evidence to suggest that the effects of social buffering, as mediated by OT, may be localized within the PVN itself. First, there are data demonstrating a functional anatomical connection in the PVN between OT-expressing neurons and CRH-expressing neurons—which regulate HPA axis activity (Vale et al., 1981; Bilezikjian and Vale, 1987). There are synaptic connections between OT- and CRH-expressing neurons, and OT from the PVN can have paracrine effects locally (Ludwig and Leng, 2006), causing alterations in neuronal firing rates following OT administration. Furthermore, the production and release of OT originates primarily from the PVN, and OTR are colocalized on CRH-expressing neurons in the PVN (Dabrowska et al., 2011). Second, from these data and others, psychosocial stress and social interactions can each promote PVN OT release (Bosch et al., 2004; Engelmann et al., 2004), which includes dendritic release from intranuclear neurons (Ludwig and Leng, 2006). Third, like social housing, local OT injections in the PVN can reduce stress-induced activation of CRH-expressing neurons and intracellular signaling in rats (Kiyokawa et al., 2004; Windle et al., 2004; Blume et al., 2008). Furthermore, in the absence of social contact, the behavioral and hormonal response to psychological stress can be attenuated by local injections of OT in the PVN (Windle et al., 2004; Blume et al., 2008), indicating that the suppressive effects of OT on the HPA axis seem to be mediated within the PVN itself. For the first time, data from this dissertation demonstrates that even in the presence of a social partner, social buffering does not occur if OT action in the PVN is inhibited by an intra-PVN injection of a selective OTR antagonist. Taken together, these data strongly support the notion that social buffering can reverse the aversive effects of psychological stress on behavior, physiology, and neurochemistry through local activation of the OT system in the PVN.

Conclusion

The socially monogamous prairie vole has emerged as a unique model system to study the neurobiological mechanisms regulating the dynamic relationship between the social environment and stress. The absence of social contact can disturb normal HPA axis activity and increase stress-related behaviors in prairie voles (Grippo et al., 2008; Lieberwirth et al., 2012). The current study demonstrates that the enduring pair bond in female-male pairs provides a source of social buffering, which may be promoted through augmented post-stress social contact similar to effects observed in humans and other gregarious species (Fraser et al., 2008; Dunbar, 2010). Furthermore, I provide direct evidence that social support recruits the OT system and that site-specific OT action in the PVN is required to promote socially-mediated stress relief in female prairie voles. Together, these data expand upon previous reports in humans that note that stress reduction through comfort from a committed partner is associated with peripheral OT release (Gordon et al., 2011), providing site-specificity and functionality to this model. Therefore, OT may serve as a common regulatory element of the social environment and the stress response, and may be a targeted agent when studying the etiology, treatment, and prevention of stress-related disorders via social buffering.

CHAPTER FOUR

GENERAL DISCUSSION, IMPLICATIONS, & FUTURE DIRECTIONS

Anxiety and depressive disorders are estimated to affect 28% and 18% of adults in the U.S., respectively, have comorbidity with other disorders, and reduce life expectancy (Kessler et al., 2005; Bosch et al., 2007; Roy-Byrne et al., 2008; Carpenter et al., 2010). In addition, the economic burden of these disorders costs $80 to $120 billion per year since the 1990's (DuPont et al., 1996; Greenberg et al., 1999; Greenberg et al., 2003). Thus, understanding the stress-induced disturbances on biological pathways may allow for improved clinical approaches toward the treatment and prevention of these disorders thereby abating their prevalence and lessening their negative impact on societal and economic stability. While increased mental health risks are concomitant with social stress, social buffering can ameliorate stress-induced biobehavioral responses and reduce the risk of subsequent mental disorders (Flannery and Wieman, 1989; Paykel, 1994; Smith et al., 1998b; Cacioppo et al., 2000; Heinrichs et al., 2003; Kikusui et al., 2006)—effects that are stronger in females than males (Olff et al., 2007). Social buffering is dependent on relationship intimacy. Therefore, support from strong social bonds, such as a romantic partner, has a greater effect in reducing the negative impact of stress (e.g., suffering from a panic disorder or psychological distress) than support from less intimate ties such as friends or relatives (Cohen and Wills, 1985; Seeman, 1996; Maulik et al., 2010). Still, the neuroendocrine mechanism through which social buffering occurs is not well understood. There is an inherent difficulty in assessing neurobiological mechanisms in humans, as well as a lack of appropriate animal models, since less than 3% of mammalian species are monogamous and display social bonding between partners (Kleiman, 1977). The studies presented in this dissertation were designed to use the monogamous prairie vole (*Microtus ochrogaster*) to development an acute stress paradigm that is sensitive to the social environment, in order to assess the impact and underlying neuroendocrine mechanisms of support by a socially bonded partner on the physiological, behavioral, and neuronal responses to a stressful event.

In Chapter 2, I found stress-specific effects on physiological and behavioral response that varied as a function of the source, intensity, and predictability of the stressor. Together, these data suggest that environmental novelty induces mild psychological stress while immobilization

and social defeat reflects more severe stress in prairie voles, similar to other rodent species (Gordon et al., 2011; Armario, 2006). Still, female prairie voles were especially sensitive to immobilization stress, modifying physiological homeostasis and normal behavioral routines, and the predictability of repeated exposures to immobilization stress modulated the adaptation to this homotypic stress. Despite being glucocorticoid resistant (Taymans et al., 1997; Hastings et al., 1999), the prairie vole hypothalamic-pituitary-adrenal (HPA) axis is still responsive to various stressors (DeVries et al., 1996; Taymans et al., 1997; Liu et al., 2001; Grippo et al., 2008; Bosch et al., 2009), social cues (Smith and Wang, 2012), and circadian cues (Taymans et al., 1997). However, little research has been done to determine whether the behavioral and physiological response in prairie voles varies as a function of the nature of the stressor. This is important as determining the responsivity of prairie voles to various stressors will lead to better models of stress in this socially and physiologically unique species. Together, these data highlight the utility of immobilization within an acute paradigm to characterize the stress response in female prairie voles.

Chapter 3 revealed that social buffering from a bonded partner can reverse the aversive effects of immobilization stress on behavior, physiology, and neurochemistry through local activation of the oxytocin (OT) system in the paraventricular nucleus of the hypothalamus (PVN). I found that immobilization stress increased anxiety-like behaviors and circulating levels of corticosterone in females recovering alone, but not the females recovering with their male partner. While stress can stimulate social seeking behaviors in humans and other gregarious mammals (Simsek et al., 2012), this did not occur in stressed female prairie voles. Rather, their male partners increased pro-social behaviors when females returned after exposure to immobilization stress. Thus, female prairie voles may experience social buffering effects through received or enacted support from their bonded partner. which can lead to a reduction in the stress response (Smith and Wang, 2012). This social buffering by the male partner was accompanied by increased OT release in the PVN. Intra-PVN OT injections reduced behavioral and corticosterone responses to immobilization stress whereas injections of an oxytocin receptor antagonist blocked the effects of the social buffering. Together, these data demonstrate that PVN OT mediates the social buffering effects on the stress response, and thus may be a target for treatment of stress-related disorders. Previous research has demonstrated the beneficial impact of social ties in adulthood on the physical, psychological, emotional, and behavioral responses

toward a stressful event (Cohen and Wills, 1985; Paykel, 1994; Smith and Wang, 2012). This current study provides evidence for a neural mechanism underlying the social buffering effect from a pair bonded partner in female prairie voles.

One issue related to the experimental design of this dissertation should be noted. As Cushing and Kramer (2005) and Morgan and colleagues (2004) noted, changes in female ovarian hormones can influence the OT and HPA system in rodents, including prairie voles. Interestingly, female prairie voles do not display a spontaneous ovulation or estrous cycle (Carter et al., 1987), though ovulation can be induced 24 hr after exposure to a male conspecific (Roberts et al., 1999). For this dissertation, female and male voles were housed in separate rooms after weaning to avoid the potential effects of exposure to male chemosensory cues on female's ovarian hormones, allowing the use of reproductively intact animals without the need for controlling the estrous cycle. While daily vaginal smears could be applied to monitor individual ovarian stages (Becker et al., 2005), repeated handling could be stressful to female subjects (Longordo et al., 2011), which would complicate the data interpretation. In addition, all female subjects in these experiments were paired with a male at the same time, a common practice in prairie vole research to synchronize changes in female ovarian hormones. Finally, all male partners were vasectomized, prior to pairing, to prevent the potential effects on female ovarian hormones through pregnancy. Thus, the impact of ovarian hormones, including estrogen and progesterone, on the OT and stress effects were controlled, but future research to evaluate the impact of OT-mediated social buffering within different stages of the estrous cycle in females.

Oxytocin-Mediated Social Buffering and Implications for Human Mental Illness

No drugs currently approved for psychiatric use directly targets or addresses the social symptoms that accompany many stress-related psychiatric conditions. Given the prosocial effects of OT, this neurohypophyseal hormone has become a target for therapeutic treatment of a wide range of psychiatric disorders, with dozens of clinical trials under way (Miller, 2013). OT gives impetus to social and sexual behavior (Carter et al., 2008), and it seems that through this action, OT can inhibit stress-induced activity in the HPA axis. In humans, social interactions can activate OT action. Specifically, when individuals engage in positive social communication with their spouse, OT levels are elevated (Holt-Lunstad et al., 2008; Gouin et al., 2010). Reciprocally,

intranasal OT administration reduces conflict-promoted cortisol response and increases positive social communication (Ditzen et al., 2009). In addition, social support can reduce stress-induced increases in cortisol levels and subjective response (e.g., calmness and anxiety), and intranasal OT administrations potentiate these effects (Heinrichs et al., 2003; Quirin et al., 2011). In rats and prairie voles, OT is released in the PVN during periods of social and sexual interaction, and this release is followed by a significant drop in the stress-induced corticosterone and anxiety-like behavior responses during acute psychological stress (male rats: Waldherr and Neumann, 2007; female prairie voles: Wsol et al., 2009). Inhibition of OT action in brain regions that release OT during psychological stress (e.g., PVN: Bosch et al., 2004; Wsol et al., 2009; CeA: Ebner et al., 2004) via site-specific or icv administration of an OT receptor antagonist can attenuate the stress-induced physiological and behavioral response (Neumann et al., 2000b; Ebner et al., 2004; Wsol et al., 2009). Therefore, it could be hypothesized that while social stress can promote psychological distress via the activation of the HPA axis, social support can attenuate these effects by marginalizing the HPA axis activity via activation of the central OT system.

One major concern for using OT as a therapeutic agent is the lack of mechanistic details and knowledge about the effects of OT in individuals in different psychological states. Several recent studies using rodent models have provided some evidence that OT may be a valuable target when treating stress-related disorders that manifest within a social context. In several socially monogamous (e.g., hamsters and prairie voles) and gregarious (e.g., rats and mice) rodent species, social isolation is met with activation of the HPA axis that promotes depression- and anxiety-like behaviors (Detillion et al., 2004; Waldherr and Neumann, 2007; Grippo et al., 2009; Norman et al., 2010; Toth and Neumann, 2013). When OT is administered to socially isolated rodents, these physiological and behavioral effects are eliminated (Detillion et al., 2004; Grippo et al., 2009; Norman et al., 2010). In kind, it seems that the positive effects of social interactions on physiology and behavior associated with distress are mediated by OT. For example, when an oxytocin receptor antagonist is administered before mating, the anxiolytic effects of mating on anxiety-like behavior in an EPM test in rats (Waldherr and Neumann, 2007). In addition, social housing can prevent the development of depression-like behavior during psychological stress in mice with chronic pain, yet blockade of the receptor-mediated action of endogenous OT inhibits the attenuation of depression-like behavior in socially-housed mice (Norman et al., 2010). Furthermore, this dissertation provides insight into the neurocircuitry that

governs the effects of OT as an acute treatment and within varying context of distress. Together, these studies support the contention that the mental health-promoting effects of social relationship are regulated by the central OT system.

Future Directions: Building a Local Circuit of Social Buffering in the PVN

Previous research has demonstrated that social interactions can be anxiolytic, and OT can act to mirror these buffering effects. From this dissertation, I provide direct evidence that post-stress social interactions with a bonded partner promote OT action in the PVN to induce socially-mediated stress relief in female prairie voles. Together, these data expand upon previous reports of OT-mediated social buffering in humans, noting comfort from a committed partner can relieve stress and promote peripheral OT release (Gordon et al., 2011), and provide site-specificity and functionality to this model. Still, in order to better understand the impact of OT in the PVN during stress recovery, it is vital to evaluate the changes to neuronal activity in the PVN as a function of OT release. In the absence of social contact, the behavioral and hormonal response to psychological stress can be attenuated by local injections of OT in the PVN (Windle et al., 2004; Blume et al., 2008), indicating that the suppressive effects of OT on the HPA axis seem to be mediated within the PVN itself. Furthermore, like social housing, local OT injections in the PVN can reduce stress-induced activation of CRH-expressing neurons and intracellular signaling in rats (Kiyokawa et al., 2004; Windle et al., 2004; Blume et al., 2008). These findings suggest that the anxiolytic effects of OT are mediate via an inhibitory effect on CRH neuronal activity; yet, the inhibitory mechanism has not been determined. It is possible that the OT acts directly on CRH-expressing neurons to modulate these neurons in the PVN (Fig. 10). In support of this hypothesis, there are data demonstrating synaptic connections between OT- and CRH-expressing neurons, OTR are colocalized on CRH-expressing neurons, paracrine release of OT that has local effects in the PVN, and altered neuronal firing rates following OT administration (Vale et al., 1981; Bilezikjian and Vale, 1987; Ludwig and Leng, 2006; Dabrowska et al., 2011). One caveat to this idea is that the majority of neuronal responses to OT are excitatory, (Inenaga and Yamashita, 1986), and thus, OT may inhibit CRH neuron indirectly.

One indirect inhibitory mechanism may include gamma-aminobutyric acid (GABA) interneuron signaling. GABA is the dominant inhibitory neurotransmitter in the mammalian

brain, with expression of local GABAergic interneurons in the PVN and PVN-projecting GABAergic neurons surrounding the PVN (peri-PVN), particularly at the dorsal part of the PVN (Herman et al., 2002; Gawronski et al., in press). Furthermore, GABA receptors, both GABAα and GABAβ, are colocalized on hypophysiotropic CRH-expressing neurons in the PVN (Herman et al., 2002), and local PVN-projecting GABAergic neurons are known to inhibit stress-induced CRH expression and neuronal firing via GABAα receptors (Bali and Kovacs, 2003; Bartanusz et al., 2004; Bali et al., 2005). Recently, it has been documented that the suppression of stress-induced CRH expression in the PVN via intra-PVN injections of OT requires activation of GABAα receptors, as co-injection of a GABAα receptors antagonists blocks OT effects. Thus, it could be postulated that the OT-mediated social buffering effects observed in this dissertation also activates local GABAergic neurons that inhibit CRH neuronal activity (Fig. 10). I am currently undertaking experiments to test the direct and indirect inhibitory mechanisms of OT on CRH neuronal activity in the PVN. For this research, I should be able to determine the neurochemical phenotype of neurons that are responsive to OT injections, during basal and stress conditions, in the PVN and whether GABAα receptor activity is required in order to observe the anxiolytic and social buffering effects mediated by OT.

APPENDIX A

FIGURES

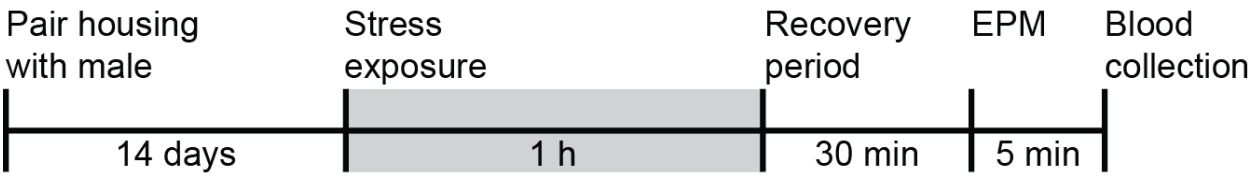

Figure 1: Testing schedule for various acute psychological and social stressors in Experiment 1.

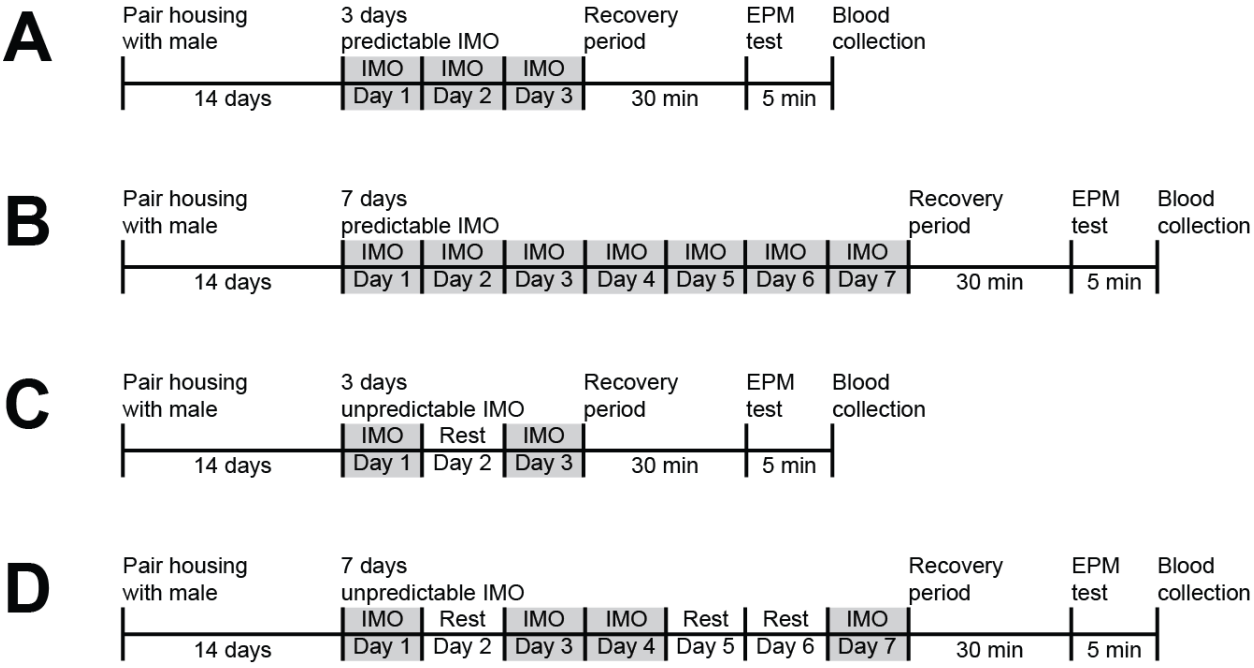

Figure 2: Testing schedule for the predictable and unpredictable immobilization treatments in Experiment 2.

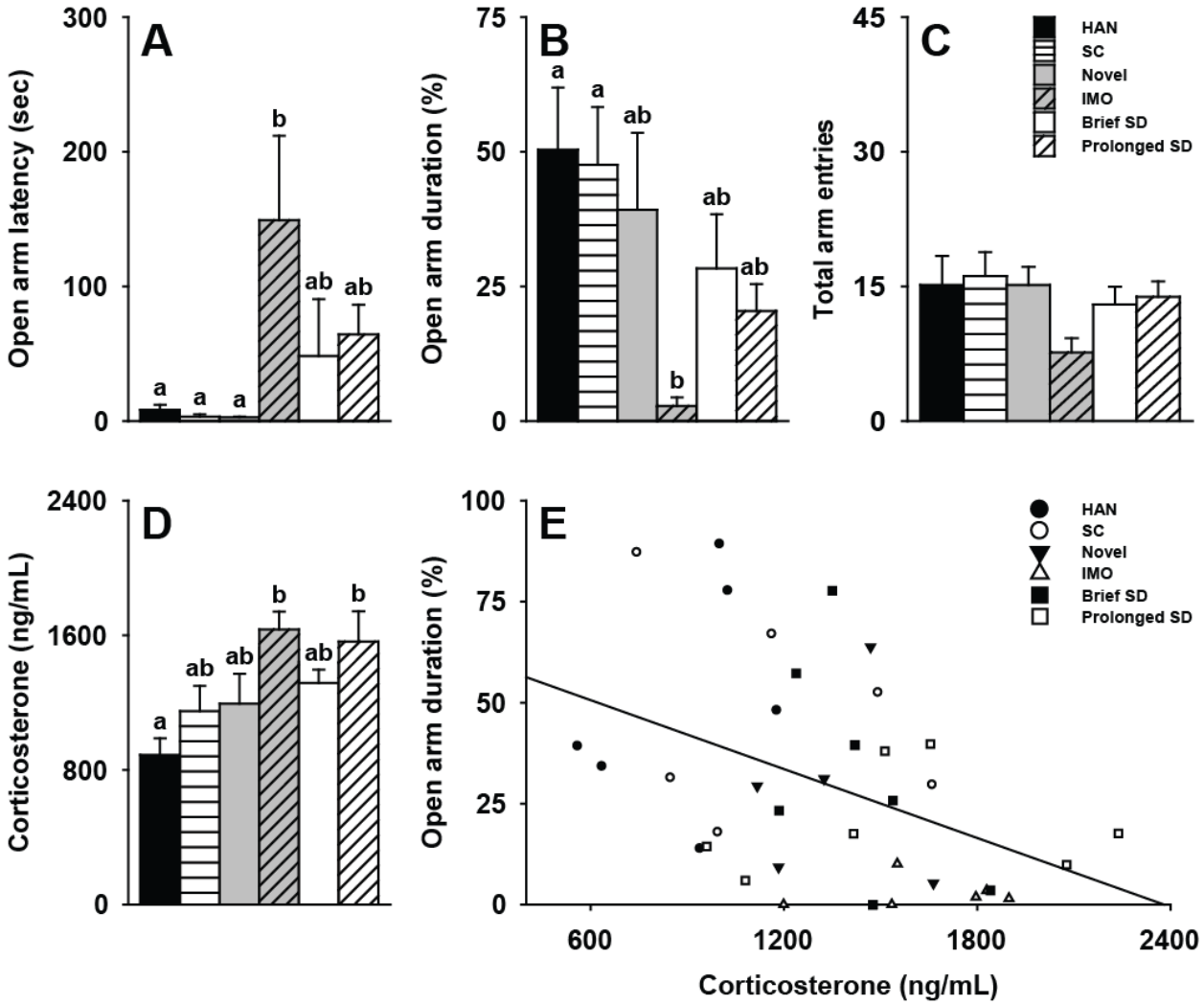

Figure 3: The source and intensity of a stressor affected the biobehavioral stress response. ***A-B***, Only immobilization (IMO) stress led to a (***A***) delay in the latency of females to enter the open arm and (***B***) decreased percentage of time that females spent in the open arms during the EPM test. ***C***, None of the stressors influenced locomotor behavior (i.e., total arm entries) during the EPM test. ***D***, Corticosterone remained elevated 30 min post-stress in response to IMO and prolonged social defeat (SD), but not environmental novelty (Novel) or brief SD, in comparison to handled controls (HAN). ***A-D,*** No differences were observed between HAN controls or social controls (SC). ***E***, Female plasma corticosterone concentrations were negatively associated with the percentage of time females spent in the open arm in the EPM test. Bars labeled with different letters differ significantly by Gabriel's *post-hoc* test in which a significant main effect was detected in the ANOVA ($p < 0.05$). ***A-D***, Data are expressed as mean ± SEM.

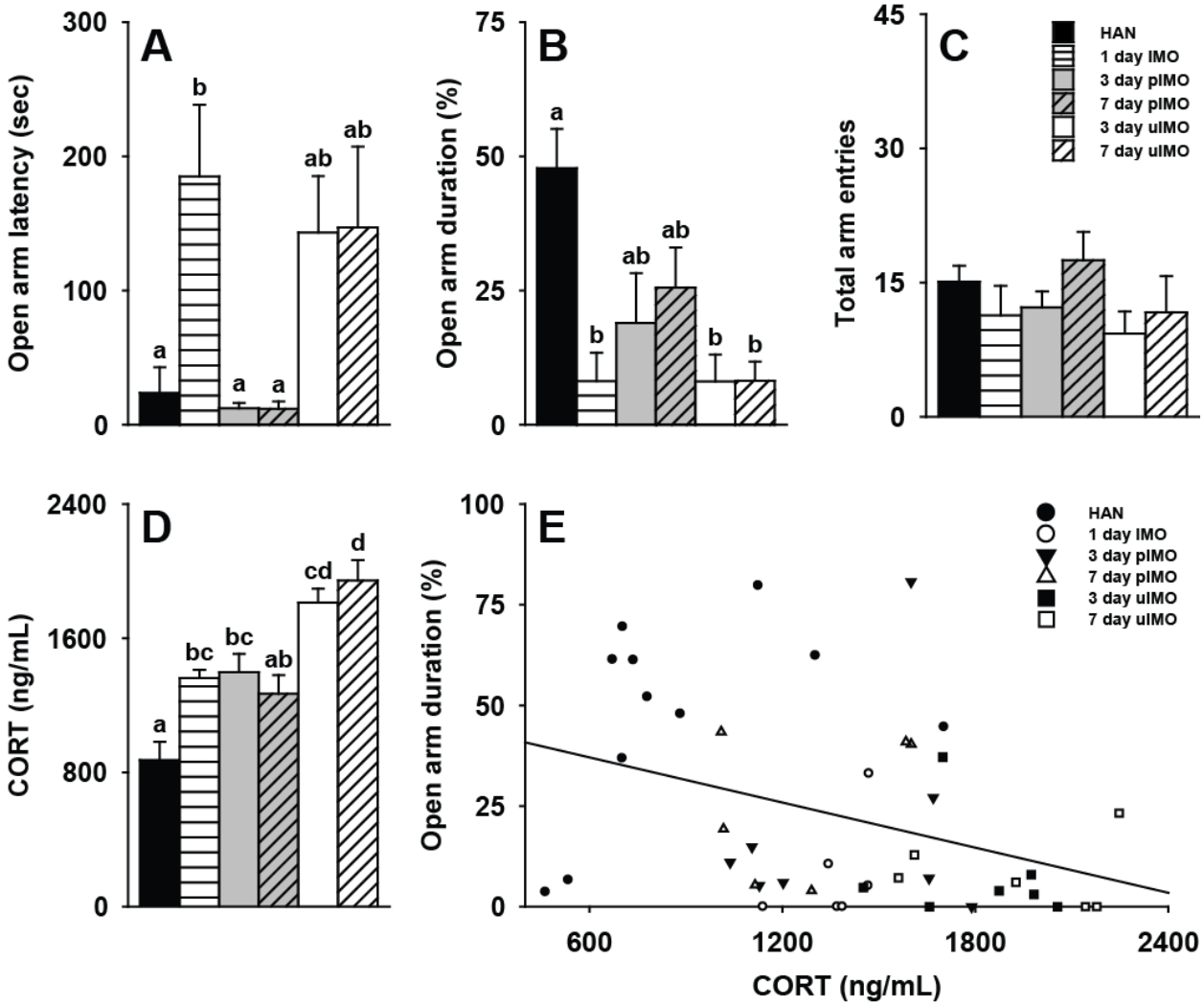

Figure 4: The predictability of immobilization (IMO) stress influenced the biobehavioral stress response. ***A***, Females exposed to 1 day IMO delayed entry into the open arm in the EPM test compared to handled control females (HAN) and females exposed to 3 or 7 day predictable IMO (pIMO). ***B***, The percentage of time that females spent in the open arms during the EPM test was lower after exposure to 1 day IMO, 3 day uIMO, and 7 day unpredictable IMO (uIMO) compared to HAN controls. No differences were observed between HAN control females or females exposed to 3 or 7 day pIMO. ***C***, None of the stressors influenced locomotor behavior (i.e., total arm entries) during the EPM test. ***D***, Corticosterone remained elevated 30 min post-stress in response to exposure to 1 day IMO, 3 day pIMO, 3 day uIMO, and 7 day uIMO compared to HAN controls. However, no differences were observed between HAN controls and 7 day pIMO. Females exposed to 7 day uIMO had significantly higher corticosterone levels than 1 day IMO and 7 day pIMO. ***E***, Female plasma corticosterone concentrations were negatively associated with the percentage of time females spent in the open arm in the EPM test. Bars labeled with different letters differ significantly by Gabriel's *post-hoc* test in which a significant main effect was detected in the ANOVA ($p < 0.05$). ***A-D***, Data are expressed as mean ± SEM.

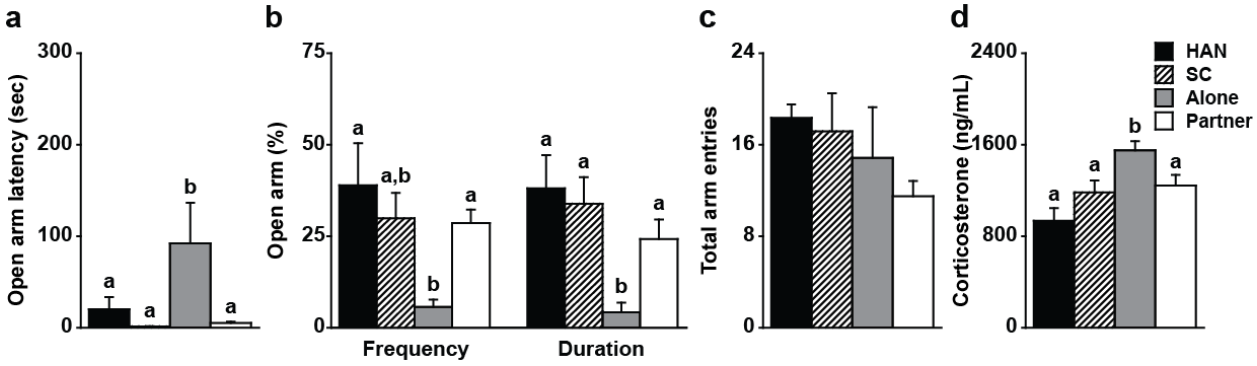

Figure 5: Social support attenuated the behavioral and hormonal stress response 30 min post-immobilization. ***A-B,*** Immobilized females recovering alone (Alone) displayed a substantial increase in EPM anxiety-like behaviors, including delayed open arm latency, fewer open arms entries, and reduced open arm duration. By contrast, females recovering with their social partner (Partner) displayed low anxiety-like behavior similar to the handle controls (HAN). ***C,*** These effects seemed to be behavior-specific as total arm entries, a locomotor measure, did not vary between groups. ***D,*** In addition to elevated EPM anxiety-like behavior, immobilized females recovering alone displayed a rise in circulating corticosterone concentrations, but females recovering with their male social partner had corticosterone levels similar to HAN controls. Social controls (SC) are non-immobilized females removed from their social partner for 30 min prior to the EPM test. Bars labeled with different letters differ significantly by *post hoc* SNK test in which a significant main effect was detected in the ANOVA ($p < 0.05$). Data are expressed as mean ± SEM.

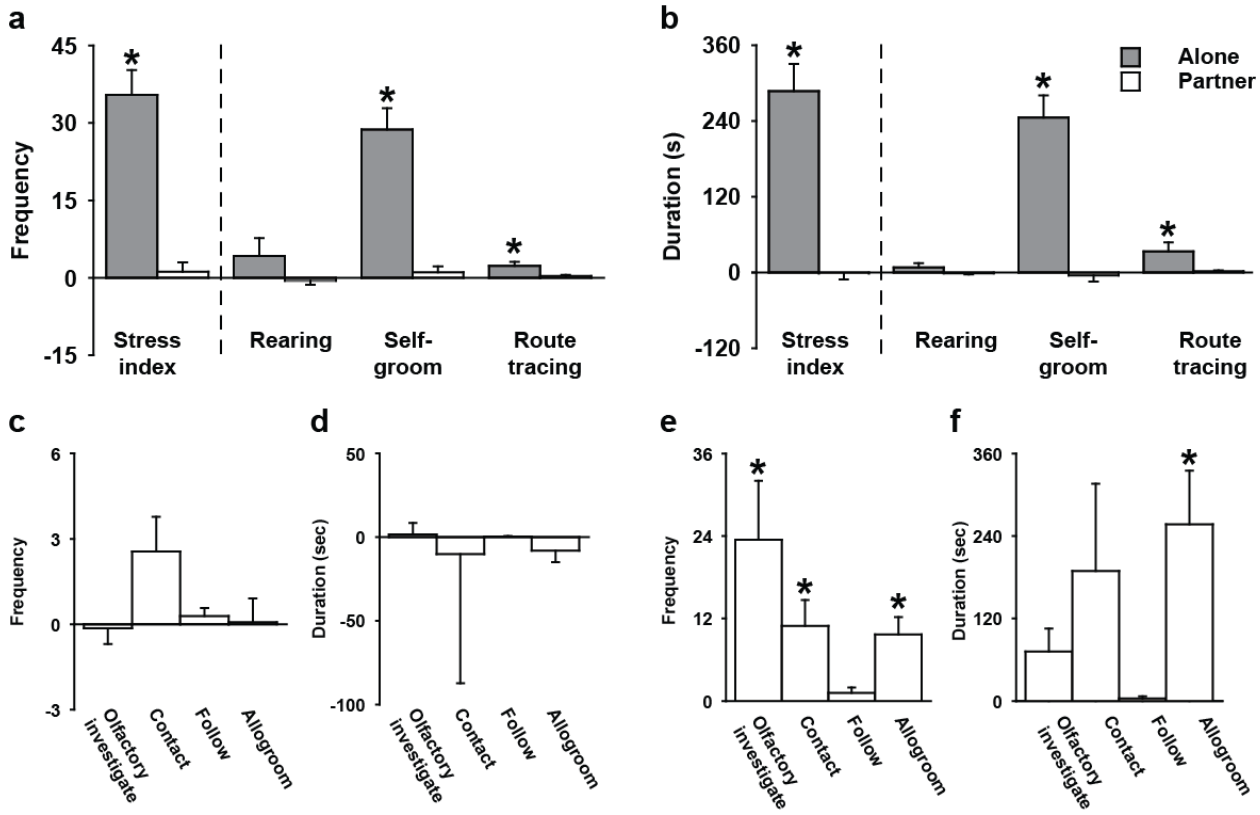

Figure 6: Social support reduced female stress-related behavior, but only males increased pro-social behavior following immobilization. ***A-B,*** Immobilized females recovering alone displayed significant increases in stress-related behavior, including rearing, self-grooming, and route tracing as well as a composite score that accounts for all stress-related behaviors (i.e., stress index), values represent a raw change score in female stress-related behaviors (post-stress minus pre-stress values). ***C-D,*** Females did not change their pro-social behavior after being immobilized, values represent a raw change score in female pro-social behaviors. ***E-F,*** Males increased their display of pro-social behaviors when their female partner returned after experiencing immobilization, values represent a raw change score in male pro-social behaviors. Bars labeled with asterisks indicate a significant change between pre- and post-stress values as determined by a one-sample or paired *t-test* ($p < 0.05$). Data are expressed as mean ± SEM.

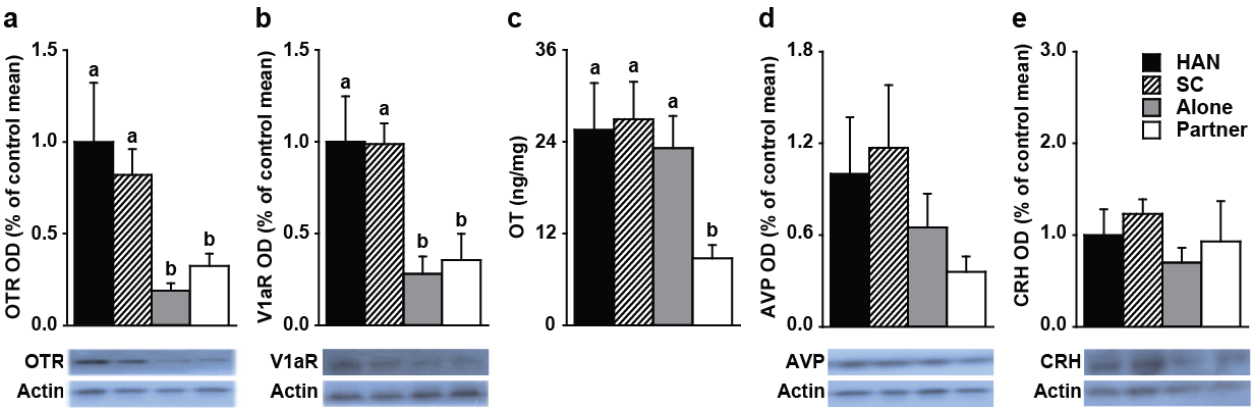

Figure 7: Neurochemical changes occurred as a result of immobilization and social support in the PVN. ***A,*** Immobilized females had significantly lower optical density (OD) of oxytocin receptor (OTR) in the PVN. ***B,*** V1aR expression was also lower in the PVN following immobilization stress. Social controls (SC) are non-immobilized females removed from their social partner for 30 min prior to sacrifice and tissue collection. ***C,*** Recovering from immobilization with a social partner induced a reduction in the oxytocin (OT) content in the PVN. ***D-E,*** No changes were observed in vasopressin (AVP) or CRH content. Bars labeled with different letters differ significantly by *post hoc* SNK test in which a significant main effect was detected in the ANOVA ($p < 0.05$). Data are expressed as mean ± SEM.

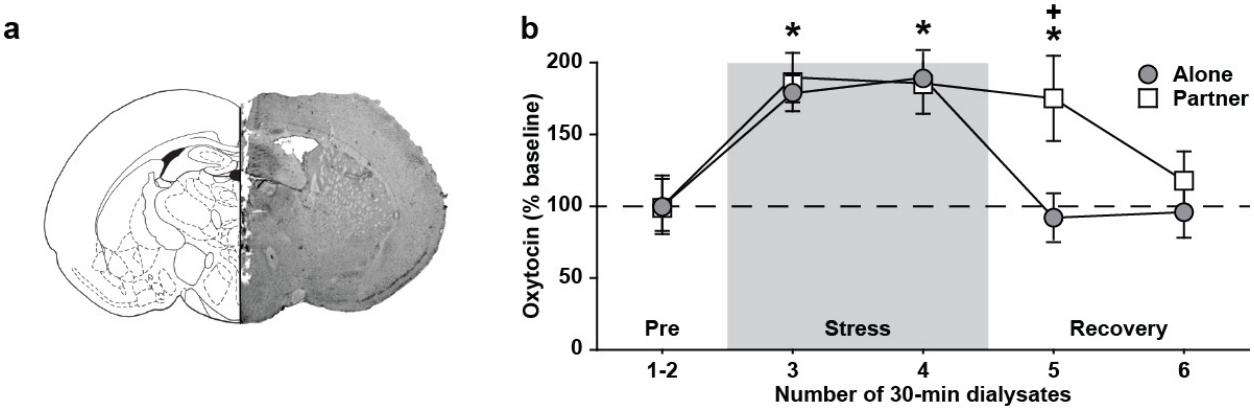

Figure 8: Social support promotes oxytocin release in the PVN. ***A,*** Schematic drawing (left) and representative photomicrograph of vole brain section (right) illustrates location of microdialysis probe placement in the PVN. ***B,*** Microdialysis was used to measure extracellular oxytocin concentrations when immobilized females recovered alone or with their social partner. Immobilization significantly increased oxytocin outflow in the PVN in all females as compared to baseline. However, during the recovery period, extracellular oxytocin concentrations only increased above baseline levels when immobilized females recovered with their social partner. Asterisks indicate differences from baseline while plus signs indicate group differences at a specific time point, determined by *post hoc* SNK test in which a significant main effect or interaction was detected in the ANOVA ($p < 0.05$). Data are expressed as mean ± SEM.

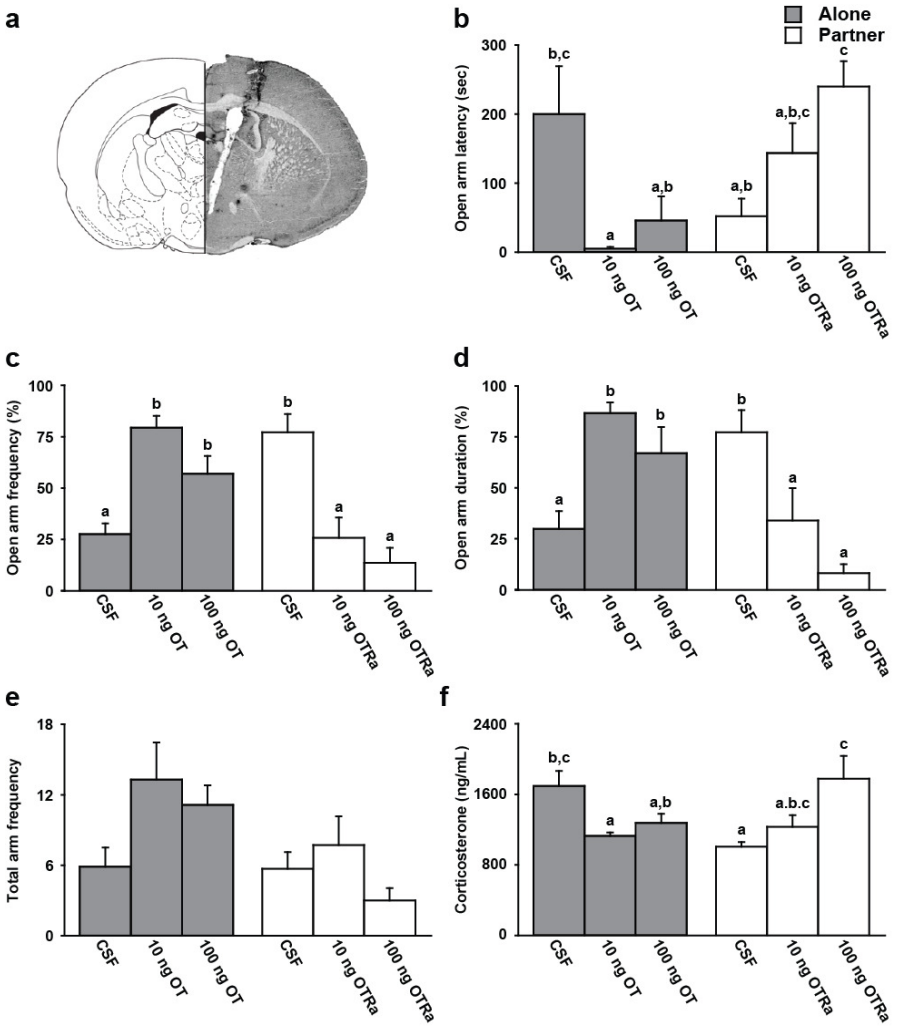

Figure 9: Oxytocin is sufficient to reduce the stress response and necessary for the social buffering effect. ***A,*** Schematic illustrations (left) and representative photomicrograph of vole brain section (right) demonstrating location of micro-injections in the PVN. All females received bilateral implantation of guide cannulae in the PVN. Females were then immobilized, injected with a vehicle (artificial cerebrospinal fluid; CSF), oxytocin (OT; 10 ng or 100 ng/200nl/side), or a selective oxytocin receptor antagonist (OTRa; 10 ng or 100 ng/200nl/side), and recovered alone or with their male partner. ***B-D,*** Females recovering alone displayed elevated EPM anxiety-like behavior unless they received an intra-PVN oxytocin injection. By contrast, females recovering with their partner displayed reduced EPM anxiety-like behavior compared to females recovering alone. When females recovering with their partner received an intra-PVN injection of a selective oxytocin receptor antagonist, no social buffering effects were observed on behavior. ***E,*** No group differences were observed in total arm entries. ***F,*** Social support and oxytocin injections reduced circulating corticosterone concentrations as compared to females recovering alone. However, when females recovering with their social partner receiving an intra-PVN selective oxytocin receptor antagonist injection, corticosterone levels were elevated, similar to females recovering alone. Bars labeled with different letters differ significantly by *post hoc* SNK test in which a significant main effect or interaction was detected in the ANOVA ($p < 0.05$). Data are expressed as mean ± SEM.

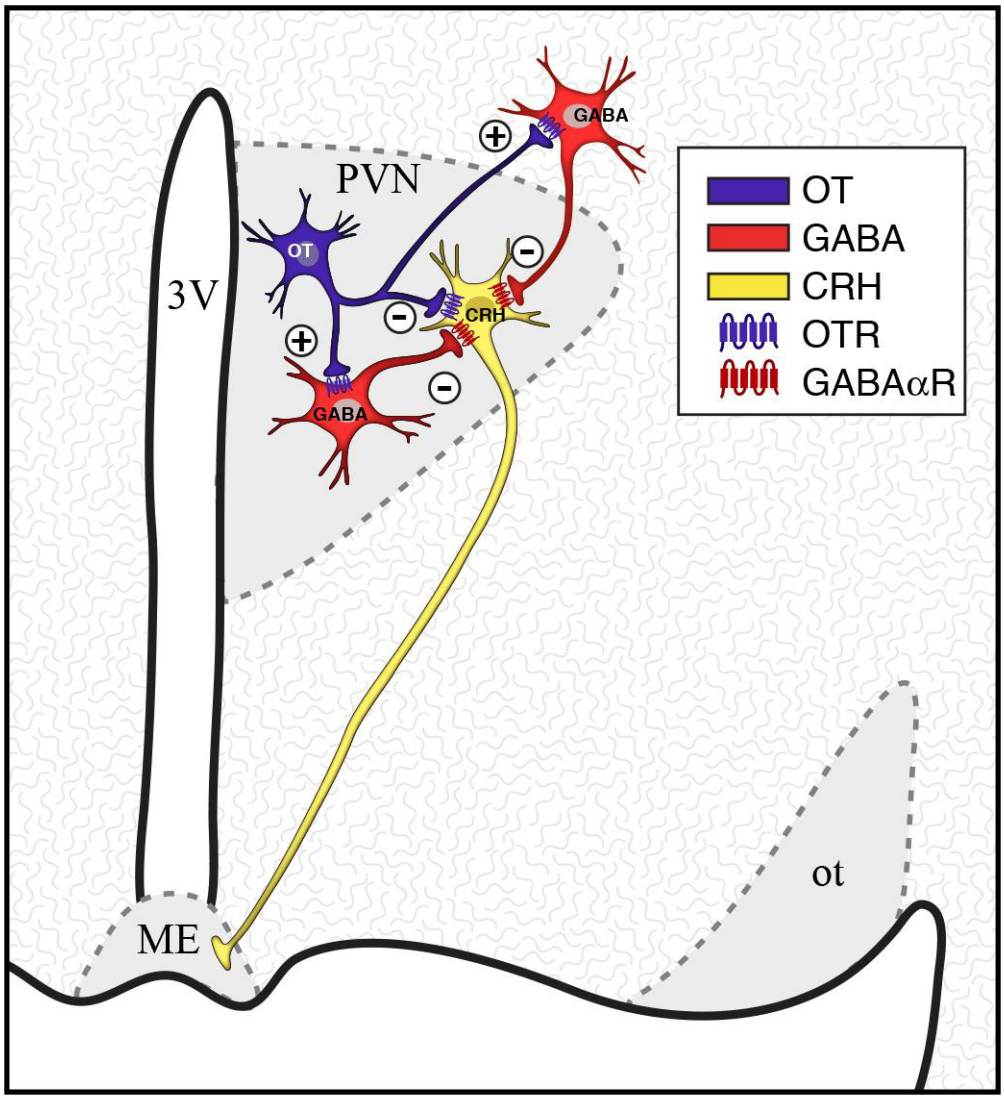

Figure 10: Model of OT-GABA regulation of PVN CRH neuron activity. Local oxytocin (OT, blue) neurons in the paraventricular nucleus of the hypothalamus (PVN) may regulate corticotrophin-releasing hormone (CRH, yellow) neuronal activity either directly or indirectly, by modulating gamma-aminobutyric acid (GABA, red) release. OTergic neurons synapse onto CRHergic neurons, which co-expression oxytocin receptors (OTR, blue), and local release of OT can reduce CRH mRNA expression and neuronal activity. In addition, OTergic neurons synapse onto PVN and peri-PVN GABAergic neurons, which co-expression oxytocin receptors (OTR), and local release of OT can increase GABA neuronal activity. Consequently, GABAergic inhibition of CRH neuron activity occurs through stimulation of GABAα receptors (GABAαR, red) co-expressed on CRHergic neurons. 3V, third ventricle; ot, optic tract; ME, median eminence.

APPENDIX B

TABLES

Table 1: Effect of stressors on the frequency of arm entries in the elevated plus maze.

Experiment 1. Various acute psychological and social stressors				
Groups	*n*	Open arm	Closed arm	% Open arm
HAN	6	5.33 ± 1.86	9.83 ± 2.24	36.59 ± 9.25
SC	6	5.33 ± 1.33	10.83 ± 1.85	30.93 ± 13.73
Novel	6	7.33 ± 1.67	7.83 ± 2.23	51.35 ± 28.09
IMO	6	1.03 ± 0.37	6.67 ± 1.65	15.83 ± 6.38
Brief SD	8	4.25 ± 1.91	8.75 ± 2.11	33.08 ± 8.89
Prolonged SD	7	3.43 ± 0.75	10.43 ± 1.17	23.99 ± 3.55
Experiment 2. Predictable vs. unpredictable immobilization				
Groups	*n*	Open arm	Closed arm	% Open arm
HAN	11	5.55 ± 1.12	9.54 ± 1.04	34.96 ± 5.69
Acute IMO	6	1.50 ± 0.72	9.83 ± 2.83	10.99 ± 6.05
3 day pIMO	8	4.13 ± 1.01	8.13 ± 1.76	35.35 ± 8.03
7 day pIMO	6	3.50 ± 1.52	14.00 ± 1.86	17.64 ± 3.97
3 day uIMO	7	2.00 ± 1.05	7.29 ± 1.74	14.15 ± 5.50
7 day uIMO	6	2.05 ± 0.86	9.67 ± 3.40	15.16 ± 6.02

Note. Values represent frequency of entries into the open and closed arms on the elevated plus maze as well as the percentage of open arm entries (open arm entries vs. total arm entries). Behaviors are reported as means and standard errors. Groups include handled controls (HAN), social controls (SC), and females exposed to novel environment stress (Novel), immobilization (IMO or acute IMO), brief social defeat (Brief SD), prolonged social defeat (Prolonged SD), 3 day predictable IMO (3 day pIMO), 7 day predictable IMO (7 day pIMO), 3 day unpredictable IMO (3 day uIMO), and 7 day unpredictable IMO (7 day uIMO).3 day uIMO

Table 2: Antibodies for Western blotting in the prairie vole brain

Primary antibody	Dilution	Incubation	MW	Secondary antibody
Mouse anti-Actin [Millipore; MAB1501]	1:10K	18-24 h	45	Bovine anti-Mouse IgG-HRP [Santa Cruz; sc-2371]
Rabbit anti-AVP [Millipore; AB1565]	1:4K	18-24 h	17	Goat anti-Rabbit IgG-HRP [Santa Cruz; sc-2030]
Rabbit anti-V1aR [Enzo; ADI-905-811]	1:100	42-48 h	48	Goat anti-Rabbit IgG-HRP [Santa Cruz; sc-2030]
Goat anti-CRF [Santa Cruz; sc-1759]	1:500	18-24 h	22	Donkey anti-Goat IgG-HRP [Santa Cruz; sc-2033]
Goat anti-OTR [Santa Cruz; sc-8102]	1:200	18-24 h	63	Donkey anti-Goat IgG-HRP [Santa Cruz; sc-2033]

Note. Molecular weight (MW) is shown in kDa. All primary antibodies were incubated in 4 °C. The dilution and incubation for all secondary antibodies were 1:10K for 1 h at room temperature (23 °C).

Table 3: Social support continued to affect female stress behavior but not female or male pro-social behavior 30 min after immobilization.

		Female stress-related behavior			
Groups	*n*	Stress index	Rearing	Self-grooming	Route tracing
Alone	6				
Frequency		10.58 ± 6.45	4.42 ± 4.59	5.08 ± 2.68	1.08 ± 0.46
Duration		32.64 ± 37.66	5.70 ± 5.91	4.80 ± 28.30	22.84 ± 11.44
Partner	7				
Frequency		-6.64 ± 4.17	-1.29 ± 1.50	**-5.79 ± 2.12***	0.36 ± 0.32
Duration		-79.43 ± 39.11	-3.70 ± 4.70	**-86.21 ± 28.18***	4.35 ± 3.81
		Female pro-social behavior			
Partner	*n*	Olf. Investigation	Contact	Follow	Allogroom
Frequency	7	-0.57 ± 0.70	-4.36 ± 5.61	0.0 ± 0.0	-0.93 ± 0.63
Duration	7	-4.08 ± 3.30	-227.10 ± 248.23	0.0 ± 0.0	-12.38 ± 10.89
		Male pro-social behavior			
Partner	*n*	Olf. Investigation	Contact	Follow	Allogroom
Frequency	7	-3.79 ± 3.93	-2.71 ± 2.54	0.07 ± 0.17	1.79 ± 1.93
Duration	7	-15.26 ± 18.46	49.79 ± 116.71	0.10 ± 0.34	96.23 ± 49.53

Note. Values represent a raw change score for each behavior (post-stress minus pre-stress values) reported as means and standard errors. Durations are measured in seconds. Groups include immobilized females recovering alone (Alone) or with a pair-bonded partner (Partner) and male partners (Partner). Asterisks indicate significant difference between pre- and post-stress values ($p < 0.05$).

Table 4: Stress and support had no effect on neuropeptide and receptor content outside of the PVN.

Region	Marker	HAN	SC	Alone	Partner	*p-value*
NAcc	CRH	1.00 ± 0.20	0.98 ± 0.16	0.90 ± 0.11	1.11 ± 0.20	0.85
	AVP	1.00 ± 0.09	1.09 ± 0.16	1.08 ± 0.19	1.40 ± 0.39	0.64
	V1aR	1.00 ± 0.32	0.94 ± 0.30	0.89 ± 0.30	1.20 ± 0.56	0.94
	OTR	1.00 ± 0.36	0.86 ± 0.23	1.02 ± 0.32	1.14 ± 0.51	0.96
MeA	CRH	1.00 ± 0.42	0.99 ± 0.36	1.15 ± 0.11	1.36 ± 0.32	0.81
	AVP	1.00 ± 0.19	0.49 ± 0.09	1.22 ± 0.25	0.83 ± 0.16	0.11
	V1aR	1.00 ± 0.19	1.42 ± 0.88	1.14 ± 0.23	0.84 ± 0.18	0.78
	OTR	1.00 ± 0.21	1.54 ± 0.97	1.32 ± 0.36	1.03 ± 0.24	0.83
CeA	CRH	1.00 ± 0.27	0.62 ± 0.16	0.73 ± 0.17	0.86 ± 0.23	0.64
	AVP	1.00 ± 0.24	0.73 ± 0.10	0.69 ± 0.04	0.84 ± 0.19	0.51
	V1aR	1.00 ± 0.10	1.29 ± 0.29	1.13 ± 0.14	1.65 ± 0.23	0.16
	OTR	1.00 ± 0.27	0.87 ± 0.19	0.71 ± 0.13	0.95 ± 0.20	0.71
SON	OT	18.46 ± 4.71	17.11 ± 1.38	16.70 ± 5.80	22.75 ± 3.91	0.62

Note. Means and standard errors are provided for each protein marker. Values for CRH, AVP, V1aR, and OTR reflect the optical density as measured via Western blotting (normalized to actin and standardized to control values). Values for OT are provided in ng/mL as measured via ELISA. Groups include handle controls (HAN), social controls (SC), and immobilization stress followed by recovery alone (Alone) or with a male partner (Partner). Oxytocin was not detectable in the NAcc, MeA, or CeA.

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Young, L.J., Young, A.Z.M., and Hammock, E.a.D. (2005). Anatomy and neurochemistry of the pair bond. *J Comp Neurol* 493**,** 51-57.

Zheng, J., Babygirija, R., Bülbül, M., Cerjak, D., Ludwig, K., and Takahashi, T. (2010). Hypothalamic oxytocin mediates adaptation mechanism against chronic stress in rats. *Am J Physiol Gastrointest Liver Physiol* 299**,** G946-G953.

BIOGRAPHICAL SKETCH

Education

Doctor of Philosophy, Neuroscience, Florida State University, expected, August 2013

Master of Arts, Psychobiology, University of Nebraska at Omaha, May 2009

Bachelor of Science, Psychology (Minor: Chemistry), *Magna Cum Laude*, University of Nebraska at Omaha, May 2007

Grant/Fellowship Support

NIH Predoctoral Ruth L. Kirschstein National Research Service Award (Principal Investigator), F31-MH-095464, "Oxytocin regulation of social buffering following stress", National Institutes of Health, 2011 – 2013, $83,947

Ruth G. and Joseph D. Matarazzo Scholarship, American Psychological Foundation/Council of Graduate Departments of Psychology, 2010, $3000

Congress of Graduate Students Grant, Florida State University, 2009 – 2011, $400

ASP Small Research Grant, American Society of Primatologists, 2009, $750

NSF Graduate Research Fellowship, "Oxytocin and pair-bond formation in marmosets", National Science Foundation, 2008 – 2011, $121,500

ASP Small Research Grant, American Society of Primatologists, 2008, $750

University Committee on Research and Creative Activity (UCRCA) Student Research Grant, Office of Sponsored Programs & Research, UNO, 2008, $500

Regents Tuition Waiver, UNO, 2007 – 2008, $2300 annual

Frank Bellinghiere Memorial Scholarship, UNO, 2006 – 2007, $650

Goodrich Scholarship, UNO, 2003 – 2007, $20,000

Superintendent Super 13 Scholarship, Omaha Public School System, 2003, $1000

Honors & Awards

90% List for 'Excellent' in Overall Assessment of Instructor (100% Rating), Department of Psychology, FSU, 2012

SfN Chapter Travel Stipend, Program in Neuroscience, FSU, 2012

Outstanding Student Paper Award, International Behavioral Neuroscience Society, 2012

Graduate Student Research and Creativity Award, Graduate School and Office of Research, FSU, 2012

IBNS Travel Award, International Behavioral Neuroscience Society, 2012

US-Japan Biology of Prosocial Behavior Workshop Travel Award, Emory University, 2011

WCNH Travel Award, World Congress on Neurohypophysial Hormones, 2011

Top 25 Hottest Articles (Rank: 1), Science Direct, Hormones and Behavior, Jan – Mar 2010

Lloyd M. Beidler Neuroscience Graduate Research Award, Program in Neuroscience, FSU, 2010

Oral Presentation Honorable Mentions, American Society of Primatologists, 2009

Dean's Scholarship, FSU, 2009

Ford Foundation Predoctoral Fellowship Honorable Mentions, National Academies, 2009

UCRCA Student Travel Award, Office of Sponsored Programs & Research, UNO, 2008

Psychology Student Travel Award, Department of Psychology, UNO, 2008

Chancellor's List, UNO, 2007

University and National Dean's List, UNO, 2005 – 2007

Publications

Research Papers: († denotes co-first authors)

Smith, A.S.†, Sun, P.†, Liu, Y., and Wang, Z. (in preparation). Breaking bonds in male prairie vole: Long-term effects on emotional and social behavior and neurochemistry.

Smith, A.S., and Wang, Z. (in review). Hypothalamic oxytocin mediates social buffering of the stress response. *Biol Psychiatry*.

Smith, A.S., Lieberwirth, C., and Wang, Z. (in press). Behavioral and physiological responses of female prairie voles to various stressful conditions. *Stress*. PMID:23647082

Birnie A.K., Hendricks S.E., **Smith A.S.**, Milam R., and French J.A. (2012). Maternal gestational androgens are associated with decreased juvenile play in white-faced marmosets (*Callithrix geoffroyi*). *Horm Behav* 62, 136-145. PMID:22705955

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French, J.A., **Smith, A.S.**, Gleason, A.M., Birnie, A.K., Mustoe, A., and Korgan, A. (2012). Stress reactivity in young marmosets (*Callithrix geoffroyi*): Ontogeny, stability, and lack of concordance among co-twins. *Horm Behav* 61, 196-203. PMID:22210196

Smith, A.S., Birnie, A.K., and French, J.A. (2011). Social isolation affects partner-directed social behavior and cortisol during pair formation in marmosets, *Callithrix geoffroyi*. *Physiol Behav* 104, 955-961. PMID:21712050

Birnie, A.K., **Smith, A.S.**, Nali, C., and French, J.A. (2011). Social and developmental influences on urinary androgen levels in young male white-faced marmosets (*Callithrix geoffroyi*). *Am J Primatol* 73, 378-385. PMID:21328596

Smith, A.S., Ågmo, A., Birnie, A.K., and French, J.A. (2010). Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, *Callithrix penicillata*. *Horm Behav* 57, 255-262. PMID:20025881

Smith, A.S., Birnie, A.K., and French, J.A. (2010). Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's marmosets, *Callithrix geoffroyi*. *Gen Comp Endocr* 166, 307-313. PMID:19854190

French, J.A., **Smith, A.S.**, and Birnie, A.K. (2010). Maternal gestational androgen levels in female marmosets (*Callithrix geoffroyi*) vary across trimesters but do not vary with the sex ratio of litters. *Gen Comp Endocr* 165, 309-314. PMID:19646445

French, J.A., **Smith, A.S.**, and Birnie, A.K. (2010). Maternal gestational androgen levels in female marmosets (*Callithrix geoffroyi*) vary across trimesters but do not vary with the sex ratio of litters. *Gen Comp Endocr* 165, 309-314. PMID:19090554

Book Chapters:

Smith, A.S., Lei, K., and Wang, Z. (in press). "Neurobiology of social attachment," in *Neurobiology of Mental Illness,* eds. D. Charney, J. Buxbaum, P. Sklar, & E. Nestler. 4th ed (New York: Oxford University Press).

Smith, A.S., Birnie, A.K., and French, J.A. (2013). "Prenatal androgens affect development and behavior in primates," in *Building Babies: Primate Developmental Trajectories in Proximate and Ultimate Perspectives*, eds. K. Clancy, K. Hinde, & J. Rutherford. (New York: Springer). 103-131.

Reviews:

Smith, A.S., and Wang, Z. (2012). Salubrious effects of oxytocin on social stress-induced deficits. *Horm Behav*, 61, 320-330. PMID:21712050

Conference Proceedings (***Presenting Author*)

***Smith, A.S.**, and Wang, Z. (2012). Immobilization stress ameliorated by oxytocin-mediated social buffering in female prairie voles (*Microtus ochrogaster*). Poster presented at the 42nd annual meeting of the Society for Neuroscience, New Orleans, LA.

***Smith, A.S.**, and Wang, Z. (2012). Social buffering requires oxytocin action in the hypothalamic paraventricular nucleus in female prairie voles (*Microtus ochrogaster*). Paper presented at the 3rd annual meeting of the Society for Social Neuroscience, New Orleans, LA.

***Smith, A.S.**, and Wang, Z. (2012). Social buffering requires oxytocin action in the hypothalamic paraventricular nucleus in female prairie voles. Poster presented at the 7th annual NIH National Graduate Student Research Conference. Bethesda, MD.

***Smith, A.S.** and Wang, Z. (2012). Paraventricular oxytocin regulates social-mediation of the stress response in female prairie voles. Poster presented at the 21st annual meeting of the International Behavioral Neuroscience Society, Kailua-Kona, HI. **Winner of Outstanding Student Paper Award**.

***Smith, A.S.**, Liu, Y., and Wang, Z. (2011). Attenuated stress response by social support: A potential role of central oxytocin. Poster presented at the 41th annual meeting of the Society for Neuroscience, Washington, DC.

***Smith, A.S.**, Lieberwirth, C., McAllister, S.L., and Saland, S. (2011). Celebrating Brain Awareness Week throughout the entire academic school year: A strategy for growth. Poster presented at the 41th annual meeting of the Society for Neuroscience, Washington, DC.

***Smith, A.S.** and Wang, Z. (2011). Stress recovery and oxytocin release in the PVN are influenced by social interactions in female prairie voles. Poster presented at the Workshop on the Biology of Prosocial Behavior at Emory University, Atlanta, GA.

***Smith, A.S.**, Birnie, A.K., and French, J.A. (2011). Social isolation affects partner-directed social behavior and cortisol during pair formation in male and female white-faced

marmosets, *Callithrix geoffroyi*. Poster presented at the 34th annual meeting of the American Society of Primatologists, Austin, TX.

***Smith, A.S.**, Liu, Y., and Wang, Z. (2011). Social support attenuates the stress response and promotes oxytocin release in female prairie voles. Poster presented at the 9th annual World Congress on Neurohypophysial Hormones, Boston, MA.

*****Birnie, A.K., **Smith, A.S.**, French, J.A., and Ågmo, A. (2011). Pair bonding in the black-pencilled marmoset (*Callithrix penicillata*): Behavioral characteristics. Poster presented at the 20th annual meeting of the International Behavioral Neuroscience Society, Steamboat Springs, CO.

***Smith, A.S.** and Wang, Z. (2011). Modulation of the stress response in female prairie voles (*Microtus ochrogaster*). Poster presented at the 9th annual FSU Psychology Graduate Research Day, Tallahassee, FL.

Lieberwirth, C., Maffeo, M., McAllister, S.L., Saland, S., and ***Smith, A.S.** (2010). Neuroscience educational outreach by the Florida State University. Poster presented at the 40th annual meeting of the Society for Neuroscience, San Diego, CA.

***Smith, A.S.**, Ågmo, A., Birnie, A.K., and French, J.A. (2010). Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, *Callithrix penicillata*. Poster presented at the 8th annual FSU Psychology Graduate Research Day, Tallahassee, FL.

***Smith, A.S.**, Ågmo, A., Birnie, A.K., and French, J.A. (2009). Oxytocin and pairing selective sociosexual behavior and social preference in marmosets, *Callithrix penicillata*. Paper presented at the 32nd annual meeting of the American Society of Primatologists, San Diego, CA. **Honorable Mentions for Outstanding Student Paper Award**.

*Birnie, A.K., **Smith, A.S.**, and French, J.A. (2009). Urinary cortisol levels and sexual behavior during pair bonding of marmosets (*Callithrix geoffroyi*). Poster presented at the 32nd annual meeting of the American Society of Primatologists, San Diego, CA.

***Smith, A.S.**, Ågmo, A., Birnie, A.K., and French, J.A. (2009). Oxytocin and pairing selective sociosexual behavior and social preference in marmosets, *Callithrix penicillata*. Poster presented at the 13th annual meeting of the Society for Behavioral Neuroendocrinology, East Lansing, MI.

***Smith, A.S.**, Ågmo, A., Birnie, A.K., and French, J.A. (2009). Oxytocin regulates the pair-bond formation and maintenance in marmosets, *Callithrix penicillata*. Paper presented at the Centennial Celebration of Student Research and Creative Activity, Omaha, NE.

***Smith, A.S.**, Birnie, A.K., and French, J.A. (2008). Maternal androgen levels during pregnancy are associated with early-life growth and aggressive behavior of offspring in Geoffroy's marmosets (*Callithrix geoffroyi*). Paper presented at the 31st annual meeting of the American Society of Primatologists, West Palm Beach, FL.

***Smith, A.S.**, Birnie, A.K., Lane, K.R., and French, J.A. (2007). Production and perception of sex differences in vocalizations of Wied's black-tufted-ear marmosets (*Callithrix kuhlii*). Poster presented at the 4th annual meeting of the Midlands Chapter for Neuroscience Grass Lecture Series, Omaha, NE.

***Smith, A.S.**, Birnie, A.K., Lane, K.R., and French, J.A. (2007). Functional significance of sexually dimorphic acoustic structures in Wied's black tufted-ear marmosets (*Callithrix kuhlii*). Poster presented at the 30th annual meeting of the American Society of Primatologists, Winston-Salem, NC

*Rukstalis, M., **Smith, A.S.**, Mozer, D.C., and French, J.A. (2006). Species-specific vocalizations in the Atlantic coastal marmosets: Structural analysis and response to playback. Poster presented at the 2006 Marmoset Research Group of the Americas conference, San Antonio, TX.

Invited talks

Smith, A.S. (2012). Stress response, social buffering, and brain oxytocin. Presented at the Neuroscience Seminar at the University of Nebraska at Omaha.

Smith, A.S. (2012). Social buffering of stress: From neurobiology to life. Keynote address at the 4th annual Student Research and Creative Activity Fair at the University of Nebraska at Omaha.

Research Experience

Graduate Student, Department of Psychology & Program in Neuroscience, Dr. Zuoxin Wang, FSU, Aug 2009 – Present

Graduate Research Assistant, Department of Psychology & Callitrichid Primate Research Center (CRC), Dr. Jeffrey French, UNO, Jun 2007 – Jul 2009

Undergraduate Research Assistant, Department of Psychology & CRC, Dr. Jeffrey French, UNO, May 2006 – Jun 2007

Part-time Laboratory Technician, Endocrine Bioservice Laboratory, Dr. Jeffrey French, UNO, May 2006 – Jun 2007

Undergraduate Research Volunteer, Department of Psychology & CRC, Dr. Jeffrey French, UNO, May 2004 – May 2006

Teaching Experience

Instructor, Conditioning and Learning Psychology Lab (EXP-3422C), Psychology Department, FSU, Fall 2012. **Achieved 100% Rating of 'Excellent' in Overall Assessment of Instructor**

Instructor, Physiological Psychology Lab (PSB3004C), Psychology Department, FSU, Spring 2012.

Guest Lecturer, Conditioning and Learning Psychology Lab (EXP-3422C), Psychology Department, FSU, Spring 2011.

Guest Lecturer, Responsible Conduct of Research (PSB-5077), Psychology Department, FSU, Fall 2010-2012

Guest Lecturer, Careers in Psychology (PSYC-2000), Psychology Department, UNO, Fall 2008 & Spring 2009

Invited Speaker, University Committee on Research and Creative Activity Brown Bag Lecture Series: Grant Writing, Office of Sponsored Programs and Research, UNO, Nov 2008

Guest Lecturer, Animal Behavior (PSYC-4270), Psychology Department, UNO, Fall 2008

Professional Activities

Professional Affiliations:

International Behavioral Neuroscience Society	Marmoset Research Group of America
American Psychological Association	Animal Behavior Society
Society for Neuroscience	American Society of Primatologists
Society of Behavioral Neuroendocrinology	International Primatological Society

Psychology National Honors Society Biology National Honors Society

Advisory Committees:

Graduate Student Advisory Committee (Treasurer), Neuroscience Graduate Student Association, Program in Neuroscience, FSU, Tallahassee, FL Aug 2012 – Jul 2013

Graduate Advisory Committee (Neuroscience Representative), Department of Psychology, FSU, Tallahassee, FL Mar 2012 – Jul 2013

Editorial/Grant/Research Review Committees:

American Society of Primatologists, Research and Development Committee, Jul 2009 – present

Ad-Hoc Grant Reviewer and Journal Referee:

L. S. B. Leaky Foundation
Ethology
Hormones & Behavior
Psychoneuroendocrinology
BMC Ecology
Frontiers in Zoology
International Journal of Psychology and Counseling

Science Education:

Judge, Science Fair, Desoto Trail Elementary School, Tallahassee, FL, Mar 2011

Campus Representative, Advocacy Coordinating Team (ACT), American Psychological Association of Graduate Students (APAGS), Aug 2010 – Jul 2013

Member/Student Presentation Judge, American Society of Primatologists Education Committee, Jul 2009 – present

Coordinator/Member, FSU Brain Awareness Program, Neuroscience Graduate Student Association Education Committee, FSU, Tallahassee, FL, Aug 2009 – Jul 2013

FSU Brain Fair, Co-founder, 2012

Friday Neuroscience Lecture & K-12 Brain Awareness Visits, Coordinator, 2010 – 2012

Mentor/Event coordinator, UNO MSLC Math & Science Mentoring Program, UNO, Omaha, NE, Jun 2008 – Jun 2009

Mentor, Summer Scholars, Office of Multicultural Affairs, UNO, Omaha, NE, Jun – Jul 2008

Cofounder, Marmoset Undergraduate Group, UNO, Omaha, NE, Mar 2007 – Aug 2009

Tutor/Mentor, HOSTS Program, Westside High School, Omaha, NE, Aug 2004 – Dec 2004

Civic Engagement:

Assistant Coach, Chiles Youth Wrestling Club, Tallahassee, FL Feb 2010 – Jul 2013

Assistant Coach, Lincoln Youth Wrestling Club, Tallahassee, FL Feb 2010 – Jul 2013

Assistant Coach, Bryan Youth Wrestling Club, Omaha, NE Nov 2008 – Mar 2009

Volunteer, Psychiatric Ward, Veteran Affair's Hospital, Omaha, NE, Aug 2005 – Jan 2006