Hypothalamic Oxytocin Mediates Social Buffering of the Stress Response

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Background: While stressful life events can enhance the risk of mental disorders, positive social interactions can propagate good mental health and normal behavioral routines. Still, the neural systems that promote these benefits are undetermined. Oxytocin is a hormone involved in social behavior and stress; thus, we focus on the impact that social buffering has on the stress response and the governing effects of oxytocin.

Methods: Female prairie voles (Microtus ochrogaster) were exposed to 1 hour immobilization stress and then recovered alone or with their male partner to characterize the effect of social contact on the behavioral, physiological, and neuroendocrine stress response. In addition, we treated immobilized female voles recovering alone with oxytocin or vehicle and female voles recovering with their male partner with a selective oxytocin receptor antagonist or vehicle. Group sizes varied from 6 to 8 voles (N = 98 total).

Results: We found that 1 hour immobilization increased anxiety-like behaviors and circulating levels of corticosterone, a stress hormone, in female prairie voles recovering alone but not the female prairie voles recovering with their male partner. This social buffering by the male partner on biobehavioral responses to stress was accompanied by increased oxytocin release in the paraventricular nucleus of the hypothalamus. Intra-paraventricular nucleus oxytocin injections reduced behavioral and corticosterone responses to immobilization, whereas injections of an oxytocin receptor antagonist blocked the effects of the social buffering.

Conclusions: Together, our data demonstrate that paraventricular nucleus oxytocin mediates the social buffering effects on the stress response and thus may be a target for treatment of stress-related disorders.

Key Words: Corticosterone, elevated plus maze, HPA axis, immobilization stress, pair-bond, social buffering

Stressful life events (e.g., divorce or death of a spouse) are deleterious to adult mental health in humans (1,2), 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 (2). In contrast, close relationships ameliorate stress-induced biobehavioral responses and reduce the risk of psychological disorders in humans (3,4). For instance, the natural occurrence of physical touch with an infant during prefeeding behaviors and nursing is sufficient to reduce the anxiety experienced by mothers during physical and psychological stress (5). This social buffering effect has also been observed through contact with a committed partner, reducing the negative impact of stress (e.g., suffering from a panic disorder or psychological distress) (3,4). Still, the neuroendocrine mechanisms underlying social buffering via committed partnerships are not well understood. Further, there is limited modeling of this phenomenon in animal research, as less than 3% of mammalian species display social bonding between partners.

The prairie vole (Microtus ochrogaster) is a socially monogamous rodent that forms long-term pair bonds between partners (6). These bonds may protect against the aversive effects of stress by attenuating the action of the hypothalamic-pituitary-adrenal (HPA) axis (7). For example, while prolonged social separation elevates basal levels of corticosterone and increases a depression-like response to acute psychological stressors (8), social pairing can reduce basal corticosterone levels (9,10). Further, reunion with a social partner can attenuate the HPA axis response associated with separation (11). Several neuroendocrine systems, including oxytocin (OT), vasopressin (AVP), and corticotrophin-releasing hormone (CRH), are involved in prairie vole pair bonds (4,6). 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 (4). In contrast, OT released from the PVN in rats acts as an anxiolytic—suppressing HPA axis function (12). Therefore, the neuroendocrine systems that are impetuses to adult social bonds may also regulate the social buffering of the stress response.

We investigated the biobehavioral effects and underlying neuroendocrine mechanisms of social buffering in female prairie voles. We established a behavioral paradigm demonstrating recovery with a male partner following immobilization stress decreases the biobehavioral stress response while promoting activity of the OT system in the PVN. We then focused on the functional role of the OT system in social buffering and demonstrated that social recovery following stress facilitates OT release in the PVN to promote social buffering.

Methods and Materials

Detailed methods and experimental design are provided in Supplement 1.

Results

Social Support Attenuated Behavioral and Hormonal Responses to Immobilization Stress

We characterized the impact of recovering with a male partner following immobilization on the behavioral and hormonal...
response in female prairie voles. Pair-bonded female voles received 1-hour immobilization, recovered either alone or with their male partner for 30 minutes, and were examined for anxiety-like behaviors in an elevated plus maze (EPM) test and circulating levels of corticosterone. Immobilized female voles recovering alone displayed a substantial increase in EPM anxiety-like behaviors, including delayed open arm latency, fewer open arm entries, and reduced open arm duration. By contrast, female voles recovering with their social partner (Partner) displayed low anxiety-like behavior similar to the handle control animals (HAN). These effects seemed to be behavior-specific, as total arm entries, a locomotor measure, did not vary between groups. In addition to elevated EPM anxiety-like behavior, immobilized female voles recovering alone displayed a rise in circulating corticosterone concentrations, but female voles recovering with their male social partner had corticosterone levels similar to HAN control animals. Social control animals (SC) are nonimmobilized female voles removed from their social partner for 30 minutes before the EPM test. Bars labeled with different letters differ significantly by post hoc Student-Newman-Keuls test in which a significant main effect was detected in the analysis of variance (p < .05). Data are expressed as mean ± SEM.

Figure 1. Social support attenuated the behavioral and hormonal stress response 30 minutes postimmobilization. (A, B) Immobilized female voles recovering alone (Alone) displayed a substantial increase in elevated plus maze (EPM) anxiety-like behaviors, including delayed open arm latency, fewer open arm entries, and reduced open arm duration. By contrast, female voles recovering with their social partner (Partner) displayed low anxiety-like behavior similar to the handle control animals (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 female voles recovering alone displayed a rise in circulating corticosterone concentrations, but female voles recovering with their male social partner had corticosterone levels similar to HAN control animals. Social control animals (SC) are nonimmobilized female voles removed from their social partner for 30 minutes before the EPM test. Bars labeled with different letters differ significantly by post hoc Student-Newman-Keuls test in which a significant main effect was detected in the analysis of variance (p < .05). Data are expressed as mean ± SEM.

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

We further evaluated the behavioral stress response by comparing the occurrence of stress-related behaviors (i.e., rearing, repetitive autogrooming, and route tracing) for 1 hour while female prairie voles remained undisturbed in their home enclosures with their male partner and again for 1 hour postimmobilization while they recovered alone or with their male partner. We observed immobilization-induced changes in stress-related behaviors in the recovery chamber compared with baseline conditions that were dependent on the social environment (stress index frequency, t11 = 4.00, p < .005; stress index duration, t11 = 3.83, p < .005; Figure 2A,B). Specifically, female prairie voles that recovered alone displayed a significant increase in these stress-related behaviors, namely route tracing (frequency, t5 = 3.07, p < .05; duration, t5 = 2.70, p < .05) and repetitive autogrooming (frequency, t5 = 4.37, p < .01; duration, t5 = 4.82, p < .005). These effects were noted during the first 30 minutes following immobilization, returning to baseline levels 30 minutes later (Table S1 in Supplement 1). We observed no changes in stress-related behaviors initially among immobilized female prairie voles recovering with their male partner (Figure 2A,B). After interacting with their male partner for 30 minutes, autogrooming behavior was suppressed (frequency, t6 = −2.73, p < .05; duration, t6 = −3.06, p < .05; Table S1 in Supplement 1). Route-tracing behavior was not different at this latter period compared with baseline (Table S1 in Supplement 1). However, this null effect may be due to the fact that seven of the eight female prairie voles 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 by immobilization and suppressed via social recovery.

In humans and other gregarious mammals, stress can stimulate social-seeking behaviors (16). Further, group members can reduce anxiety by increased prosocial behaviors—behavior that is performed for the benefit of others—directed toward distressed individuals (4). In the current study, we did not observe a substantive change in female social behaviors after immobilization (Figure 2C,D; Table S1 in Supplement 1). However, male prairie voles augmented their social behaviors during the first 30 minutes when their female partner returned from immobilization—approaching (frequency, t6 = 2.91, p < .05), sniffing (frequency, t6 = 2.74, p < .05), and grooming (frequency, t6 = 3.80, p < .01; duration, t6 = 3.31, p < .05) the female more often (Figure 2E,F). Such effects were not observed in the subsequent 30 minutes (Table S1 in Supplement 1). Only one of the eight pairs was observed mating during the prestress and poststress periods. Therefore, as female prairie voles recovered from immobilization with their male partner, they experienced an enhanced social display from their male partner and concomitantly displayed less stress-related behaviors.

Immobilization Affected Neuropeptide Receptor Content in a Brain Region-Specific Manner

Western blotting revealed the 1-hour immobilization reduced the content of OT receptors (OTR) (F3,19 = 5.37, p < .01;
Figure 2. During the initial 30 minutes of recovery after immobilization, social support reduced female stress-related behavior, but only male voles increased social behavior following immobilization. (A, B) Immobilized female voles 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 (poststress minus prestress values). (C, D) Female voles did not change their social behavior after being immobilized; values represent a raw change score in female social behaviors. (E, F) Male voles increased their display of social behaviors when their female partners returned after experiencing immobilization; values represent a raw change score in male social behaviors. Bars labeled with asterisks indicate a significant change between prestress and poststress values as determined by a one-sample or paired t test \((p < .05)\). Data are expressed as mean \pm SEM. Alone, immobilized female voles recovering alone; Partner, female voles recovering with their social partner.

Figure 3A) and AVP V1a receptors (V1aR) \((F_{3.9} = 5.88, p < .05; \text{Figure 3B})\) 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 and central nuclei of the amygdala (CeA), indicating that the effect was brain region-specific (Table S2 in Supplement 1). Data from other rodent species have shown that stress can increase OT and AVP release in the PVN (17), and persistent OT and AVP release can lead to receptor desensitization and internalization within 30 min (18,19). Subsequently, internalized OT receptors undergo endocytosis and some V1aRs are not recycled back to the cell surface (20). Therefore, decreased OTR and V1aR expression in the PVN may indicate immobilization-induced activation of both systems.

OT Was Released in the PVN During Immobilization and Social Buffering

We measured the total content of OT via enzyme-linked immunosorbent assay, as well as AVP and CRH via Western blotting in the PVN (Figure 3C-E), NAcc, medial nuclei of the amygdala, and CeA (Table S2 in Supplement 1). Immobilized female prairie voles that recovered with a male partner had a significantly lower level of OT in the PVN compared with other groups \((F_{3.22} = 4.47, p < .05; \text{Figure 3C})\). As OT content was not detectable in the NAcc or amygdalar nuclei, we also measured OT in the supraoptic nucleus of the hypothalamus (Table S2 in Supplement 1) for a control region. No group differences were found in the OT, AVP, or CRH content in any of the other brain regions measured (Figure 3C-E; Table S2 in Supplement 1). Therefore, the social buffering-induced decrease in the PVN OT was brain region-specific 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 messenger RNA expression in the PVN is significantly increased 30 minutes following immobilization (21), and social contact facilitates OT activity (22). Therefore, a decrease in PVN OT content following immobilization and subsequent social buffering may be more likely due to a change in OT release. We used brain microdialysis with enzyme-linked immunosorbent assay to measure extracellular OT concentrations in the PVN in immobilized female voles that recovered alone or with their male partner (Figure 4A). As compared with baseline sample concentrations, immobilization significantly increased OT concentrations in the PVN in all female voles \((F_{3.44} = 24.76, p < .0001; \text{Figure 4B})\), similar to reports in rats (23,24). When immobilized female voles recovered alone, PVN OT concentrations returned to baseline levels, demonstrating no lasting release associated with the stress. Intriguingly, when female voles recovered with their male partner, PVN OT...
interaction was detected in the analysis of variance (ANOVA) in the prefrontal cortex (PFC) of female vole brains. Social control animals (SC) were non-immobilized female vole brains recovered alone, indicating that OT is anxiolytic. In contrast, intra-PVN injections of an OTR antagonist increased anxiety-like behaviors and reduced circulating levels of corticosterone in female vole brains recovering alone, indicating that OT is anxiolytic.

Figure 4. Social support promotes oxytocin release in the paraventricular nucleus (PVN). (A) Schematic drawing (left) and representative photomicrograph of vole brain section (right) illustrate location of microdialysis probe placement in the PVN. (B) Microdialysis was used to measure extracellular oxytocin concentrations when immobilized female vole brains recovered alone or with their social partner. Immobilization significantly increased oxytocin outflow in the PVN in all female vole brains as compared with baseline. However, during the recovery period, extracellular oxytocin concentrations only increased above baseline levels when immobilized female vole brains 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 Student Newman-Keuls test in which a significant main effect or interaction was detected in the analysis of variance (p < .05). Data are expressed as mean ± SEM. Alone, immobilized female vole brains recovering alone; Partner, female vole brains recovering with their social partner. (Schematic reprinted from Paxinos and Watson [50], with permission from Elsevier, copyright 1998.)

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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 (3,4). The current study provides evidence for a neural mechanism underlying the social buffering effect from a partner in pair-bonded female prairie voles. Female voles displayed buffered anxiety-like behavior and levels of corticosterone when recovering from psychological stress with their male partner. Furthermore, OT was released in the PVN when immobilized female voles recovered with their male partner, and released OT mediated the social buffering effects on biobehavioral responses to stress. Together, these data demonstrate that PVN OT is one mechanism through which social buffering occurs in female prairie voles.
Social Buffering: An Impetus for Social Living

The driving forces that facilitate social attachments, such as pair bonding, include reinforcing social behaviors through activating the brain reward centers, the consequences of disruption or separation of close social bonds on an individual's well-being and emotional state, and the beneficial effects of close social contact to improve mental and physical health (26). Previous research has documented that pair bonding in prairie voles is 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 pair bonds in prairie voles (6). 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 (14,15). Moreover, prolonged social separation from a bonded partner can elevate basal levels of corticosterone in circulation, CRH messenger RNA expression in the brain, and depression-like behavioral response to acute psychological stressors in prairie voles (8). The current study focuses on social buffering and demonstrates that social support attenuates the stress response as the immobilization-induced increase in stress-related behaviors and circulating corticosterone levels were eliminated when female voles recovered with their male partner. This extends results from previous research showing that social housing with a male prairie vole, but not a female prairie vole, can reduce the baseline HPA axis activity in female prairie voles (9). Therefore, interactions with a male partner seem to alleviate stress and benefit the emotional state and well-being of female prairie voles that live in a fundamentally social environment.

Researchers have found that experience of a stressful event can attract group members and social partners together (16), and this can lead to a reduction in their stress levels (4). For example, rats and humans are more likely to affiliate when under duress 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 we did not observe a substantive change in social-seeking behavior in female prairie voles after experiencing psychological stress, their male partners augmented their social behavior. Particularly, after olfactory investigation, male prairie voles established contact with the female and engaged in social grooming. From rats to humans, individuals will act to facilitate to extinguish distress in another (27). Consoling distressed individuals following social conflicts seems to be a critical behavior for stress coping in greater apes (28). Furthermore, 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 (29). 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 and Potential Therapeutic Benefits

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 social bond-promoting behaviors and their buffering effects on the stress response. The beneficial effects of social interaction on stress reduction seem 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 (30), which, in turn, are correlated with decreased levels of blood pressure and cardiovascular activity (29). In male rats, mating releases OT in the PVN and facilitates a reduction in anxiety-like behavior during psychological stress (31). Our data depict a similar story, as social recovery promotes the release of OT in the PVN while reducing the stress response in female prairie voles. However, unlike male rats (31), the OT release in the PVN in female prairie voles seems to be independent of sexual interaction, as such OT release was significantly elevated in all eight female voles recovered with the male partner, although only one dyad engaged in mating behavior. Sexual encounters have been demonstrated to evoke OT release in female prairie voles [e.g., NAcc (32)]; 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 prefeeding behaviors and nursing is sufficient to evoke OT release in newborn rat pups (33) and postpartum female rats (34), which is thought to originate from centrally projecting PVN neurons (35). In our study, male partners established side-by-side contact with and groomed the female voles more often after the female voles returned from the immobilization. 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 pharmacologic 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 (31,36). Our data demonstrate that despite recovering with a social partner after psychological stress, female prairie voles do not display stress relief if concomitantly receiving an injection of a selective OTR antagonist. 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 (37) and CeA (38)] can attenuate the stress-induced biobehavioral response (38). Thus, our findings support the notion that OT is one mechanism that propagates social buffering.

No drugs currently approved for psychiatric use directly target or address the social symptoms or components that accompany many stress-related psychiatric conditions. Given the prosocial effects of OT, this neurohypophysial hormone has become a target for therapeutic treatment of a wide range of psychiatric disorders, with dozens of clinical trials underway (39). Oxytocin gives imetus to social and sexual behavior (40), 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, OT levels are elevated when individuals engage in positive social communication with their spouse (41,42). Reciprocally, intranasal OT administration reduces the conflict-promoted cortisol response and increases positive social communication (43). Further, 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 (25,44). Still, before OT can be normally prescribed as
a therapeutic agent, more knowledge is necessary regarding 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. Analogous to social support, OT appears to function as an anxiolytic agent. Social isolation activates the HPA axis and promotes depression-like and anxiety-like behaviors in socially monogamous (e.g., hamsters and prairie voles) and gregarious (e.g., rats and mice) rodents (14,15,31,36,45). By contrast, OT administered during periods of social isolation can inhibit many of these negative effects (14,15,36,45). 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 (14). Our data demonstrate in the absence of a social partner, 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 without social contact.

Another major concern for clinical use of OT is the lack of mechanistic details. 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. 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. There are synaptic connections between OT-expressing and CRH-expressing neurons, and OT from the PVN can have paracrine effects locally (46), causing alterations in neuronal firing rates following OT administration. The production and release of OT originates primarily from the PVN, and OTR is co-localized on CRH-expressing neurons in the PVN (47). Second, from our data and others, psychosocial stress and social interactions can promote PVN OT release (17,37), which includes dendritic release (46). Third, like social housing, local OT injections in the PVN can reduce stress-induced activation of CRH-expressing neurons and intracellular signaling in rats (48,49). 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 (48,49), indicating that the suppressive effects of OT on the HPA axis seem to be mediated within the PVN itself. For the first time, our data demonstrate 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.

Conclusions

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 (14,15). 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 (28,29). Furthermore, we 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, our data expand upon previous reports in humans that note stress reduction through comfort from a committed partner is associated with peripheral OT release (30), 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.

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