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Anxiety-like behavior and neuropeptide receptor expression in male and female prairie voles: the effects of stress and social buffering

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Abstract

Strong social support can negate negative health outcomes – an effect defined as ‘social buffering’. In the present study, using the socially monogamous prairie vole (*Microtus ochrogaster*), we examined whether the presence of a bonded partner during a stressful event can reduce stress responses. Adult, pair-bonded female and male voles were assigned into experimental groups that were either handled (Control), experienced a 1-hr immobilization (IMO) stress alone (IMO-Alone), or experienced IMO with their partner (IMO-Partner). Thereafter, subjects were tested for anxiety-like behavior, and brain sections were subsequently processed for oxytocin receptor (OTR) and vasopressin V1a-type receptor (V1aR) binding. Our data indicate that while IMO stress significantly decreased the time that subjects spent in the open arms of an elevated plus maze, partner’s presence prevented this behavioral change – this social buffering on anxiety-like behavior was the same for both male and female subjects. Further, IMO stress decreased OTR binding in the nucleus accumbens (NAcc), but a partner’s presence dampened this effect. No effects were found in V1aR binding. These data suggest that the neuropeptide- and brain region-specific OTR alterations in the NAcc may be involved in both the mediation and social buffering of stress responses. Some sex differences in the OTR and V1aR binding were also found in selected brain regions, offering new insights into the sexually dimorphic roles of the two neuropeptides. Overall, our results suggest a potential preventative approach in which the presence of social interactions during a stressor may buffer typical negative outcomes.

Keywords

Immobilization stressor; OTR; V1aR; NAcc; sex difference; social buffering

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Conflict of interest

The authors have no financial disclosures and have no potential conflicts of interest.

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1. Introduction

Humans, as social creatures, have consistently relied on social bonds to ensure survival and success. Social environments can have a large impact on human health and can influence negative outcomes including anxiety, depression, hypertension, and other similar comorbidities [1–3]. The presence of strong, social bonds in one's environment can provide protective effects against negative health outcomes, whereas the loss of a close bond can have opposing, detrimental effects [4, 5]. The protective ability of social bonds to dampen or 'buffer' various negative health outcomes that may arise has been an up and coming area of research. These protective effects have been coined the 'social buffering' effect, and the impacts can vary by strength of the bond (i.e. partner versus friend), age, and sex [6–8]. Although previous studies have provided multiple examples of how social relationships and interactions may buffer negative health outcomes, the underlying mechanisms still remain largely unknown. Recently, efforts have been made in examining the underlying neural mechanisms of social buffering through the use of animal models, but these have been limited mainly due to most traditional animal models lacking the complex social structures seen in humans [9, 10]. Thus, additional research using alternate, more optimal animal models for the study of social buffering is necessary.

The prairie vole (*Microtus ochrogaster*), a rodent species common in the grasslands of the United States, has been established as an animal model for the examination of the neurobiology of social behaviors due to its unique life strategy of social monogamy [11–15]. Upon twenty-four hours of mating, male and female prairie voles form robust preferences for their partners (i.e. partner preference), which has been shown to last for weeks [12, 16–18]. Given their ability to form stable and enduring pair bonds, the prairie vole offers an excellent opportunity to study how strong, specific social bonds may play a role in the social buffering of stress responses [9, 19, 20]. Data from a recent study demonstrates that the presence of a male, pair-bonded partner during the recovery period following a 1-hour immobilization (IMO) stress dampens the negative effects that typically arise if the stressed female prairie vole recovers alone. Further, these effects are mediated by oxytocin (OT) in the paraventricular nucleus (PVN) of the hypothalamus [20]. Data from another study in prairie voles also confirms that consolation behaviors from pair-bonded partners toward the stressed mate can reduce the normal stress response of the mate. These consolation behaviors are also mediated by OT, but in this case, in the anterior cingulate cortex (ACC) [19].

Indeed, OT has been shown to play a critical role both for general social behaviors [21, 22] and for the buffering of stress in humans [23–26] and other traditional animal models [27–30]. Along with OT, a similar neuropeptide differing in only two amino acids, arginine vasopressin (AVP), has been implicated in general social behaviors [31–33] as well as in pair bonding behaviors of prairie voles [9, 12]. The OT receptor (OTR) and AVP V1a-type receptors (V1aR) distributions have also been mapped in various species of voles [34, 35]. Striking species differences and some subtle sexual dimorphisms have been found in the OTR and V1aR expression in the brain, implicating their potential roles in species- and sex-specific cognitive and behavioral functions [16, 35–38]. Given the known sexually dimorphic roles of these two neuropeptides in other social behaviors, [39–44] as well as the

involvement of OT in social buffering behaviors in prairie voles [9, 10, 19, 20, 45], a further exploration of both OT and AVP alterations in social buffering effects, along with any potential sex differences, is essential. Further, although previous studies have demonstrated the ability of social bonds to dampen anxiety-like behaviors during recovery from a stressor in prairie voles [19, 20], the question of whether these same anxiety-like behaviors can in fact be prevented in the first place by providing social support during the stress has yet to be examined. Therefore, in the present study, we integrated these unanswered questions to examine the potential social buffering effects from the presence of a pair-bonded partner during IMO stress on anxiety-like behaviors and OTR/V1aR expression in the brain in both male and female prairie voles. We hypothesized that male and female voles may differ in their responses to the IMO stressor as well as to the partner's social buffering effects on stress responses, and that these differences would correlate with sexual dimorphic alterations in OTR and V1aR expression in the brain.

2. Materials and Methods

2.1 Subjects

Subjects were male and female prairie voles (*M. ochrogaster*) captive-bred at Florida State University. All voles were weaned on postnatal day 21 and housed in Plexiglas cages (29×18×13 cm) with a same-sex conspecific. All cages contained cedar chip bedding with food and water provided *ad libitum*. All subjects were kept at 20°C under a 14:10 h light:dark cycle (lights on at 0700). At the time of testing, subjects had reached adulthood (70–90 days of age) and were sexually naïve before pairing occurred. Male subjects were paired with unrelated, ovariectomized female partners for two weeks, whereas female subjects were paired with unrelated, vasectomized males for two weeks to allow for the development of the stable pair bond [16–18, 46]. All vasectomized and ovariectomized animals were allowed a two-week recovery period before pairing. Subjects were randomly assigned into one of three experimental groups that were either handled (Control; 16 males and 16 females), experienced a 1-hr immobilization (IMO) stress alone (IMO-Alone; 14 males and 13 females), or with their partner (IMO-Partner; 17 males and 16 females). Behavioral testing was performed at the two-week mark for all subjects. All procedures were approved by the Institutional Animal Care and Use Committee at Florida State University and were in accordance with the guidelines set forth by the National Institutes of Health.

2.2 Immobilization Stressor (IMO)

The immobilization (IMO) test was conducted in a clean Plexiglas cage (20×25×45 cm) with fresh cedar chip bedding. The IMO stressor consisted of a plastic centrifuge tube (50 mL) with the top 1/3rd cut off to adjust for size. This restraint has been adapted from previous studies in which it has been confirmed to elicit physiological and behavioral stress responses in prairie voles [20, 47]. The front tip of the centrifuge tube was also cut off to allow the subject better respiration. After the subject was placed inside of the tube, a piece of plastic mesh was secured on top of the cut-off side of the tube with Velcro straps in order to completely immobilize the subject. Once in the IMO tube, the subject was placed into the cage and randomly assigned into one of the two treatment groups: subjects left immobilized either alone (IMO-alone) or with their free moving partner, who was placed into the cage for

one hour (IMO-partner). Non-stressed subjects were placed into a clean cage with their partners as a baseline control for handling and novelty stimulation. All subjects placed into cages with partners were recorded via Active Webcam Software (<http://www.pysoft.com/activewebcammainpage.htm>) for the entire hour.

2.3 Elevated Plus Maze (EPM)

The elevated plus maze (EPM) has been established and validated in previous vole studies [47–49]. Briefly, the apparatus is elevated 45 cm off the ground and consists of two open (35×6.5 cm) and two closed arms (35×5×15 (H) cm) that cross in the middle. The light intensity 30 cm above the center of the EPM apparatus was approximately 150 lux. After five minutes of habituation in a clean cage upon removal from the IMO, subjects were placed onto the center of the maze facing an open arm. A piece of cardboard was placed in front of the subject in order to ensure that the subject did not attempt to immediately run off the maze. Subject's behaviors were recorded for 5 minutes using Active Webcam software and were subsequently quantified by an observer blind to treatment via J-Watcher (<http://www.jwatcher.ucla.edu/>). Behaviors quantified included the latency to enter the open arms as well as frequency and duration in the open or closed arms. The percentage of time in the open arms and locomotor activity (as indicated by the total number of arm entries) were calculated.

2.4 Brain Tissue Preparation

All subjects were decapitated immediately after EPM testing (i.e. within five minutes after EPM testing). Brains were collected and placed on dry ice. All brains were stored at –80 °C until processing. Coronal brain sections were sliced for all subjects at 14 µm and mounted onto Superfrost Plus slides in a 1:8 series (at 98 µm intervals) to be stored at –80 °C until autoradiographic processing.

2.5 Receptor Autoradiographic Binding

Alternate sets of brain sections were processed for OTR and V1aR autoradiographic binding, respectively, using an established method [36, 38]. All slides were placed at room temperature 30 minutes before beginning the receptor binding processing. Brain sections were washed twice, each for 10 minutes, in 50 mM Tris-HCL (pH 7.4). All brain sections were then incubated in a trace buffer containing 50 mM Tris-HCL (pH 7.4), 10 mM MgCl₂, 0.4% BSA, 0.05% bacitracin, and 50 pM ¹²⁵I-LVA for V1aR or (PerkinElmer NEX 310) 50 pM ¹²⁵I-OVTA for OTR (PerkinElmer NEX 254) for 90 minutes at room temperature. After the 90-minute incubation, all sections were dipped in incubation buffer to remove excess tracer. Sections were then fixed in prechilled 0.1% paraformaldehyde in Tris-HCL with 10mM MgCl₂ for 4 minutes, and then washed in chilled Tris-HCL with 10 mM MgCl₂ four times for 5 minutes each. Sections were then placed in chilled Tris-HCL with 10 mM MgCl₂ for 30 minutes with stirring. After the 30-minute stirring, all sections were dipped in ddH₂O twice and dried under a stream of cool air. Dried sections were covered with a BioMax MR film (Kodak) with I microscale (Amersham, Chicago, IL) for 2 (V1aR) or 4 (OTR) days. Binding density was quantified via ImageJ software (<https://imagej.net/Welcome>). For each brain region, the density was quantified by averaging the density in three consecutive slices of the brain region for each specific region measured. Regions measured included the

prefrontal cortex (PFC), ventromedial hypothalamus (VMH), lateral septum (LS), bed nucleus of stria terminalis (BNST), amygdala (AMY), and nucleus accumbens (NAcc) for their previously known involvement in social and/or anxiety-related behaviors [9, 10].

2.6 Data Analysis

All behavioral and receptor binding data were analyzed by two-way ANOVA (group by sex) using IBM SPSS Statistics 19 software (SPSS, Inc., an IBM Company). Significant group differences were further examined by the Student-Newman-Keuls posthoc test.

3. Results

3.1 IMO stressor and partner presence affected anxiety-like behavior

The anxiety-like behaviors were measured by an EPM test following the IMO stress. Our data showed no group differences in the latency to enter the open arms (Figure 1a). However, the IMO stressor significantly decreased the frequency of subjects' entries to the open arms of the EPM in both the IMO-alone and IMO-partner groups ($F_{(2,86)} = 16.670$, $p < 0.01$; Figure 1b). No overall sex differences ($F_{(1,86)} = 1.854$, $p > .05$) or sex by treatment interactions ($F_{(2,86)} = 0.170$, $p > .05$) were seen. The IMO stressor also altered the percentage of the time that subjects spent in the open arms ($F_{(2,86)} = 9.456$, $p < 0.01$), and the posthoc test indicated that IMO-alone subjects spent less time in the open arms compared to the other two groups which, in turn, did not differ from each other (Figure 1c). No overall sex differences ($F_{(1,86)} = 0.032$, $p > .05$) or sex by treatment interactions ($F_{(2,91)} = 1.058$, $p > .05$) were seen. Finally, the IMO stressor also affected subjects' overall locomotor activity – both IMO-alone and IMO-partner groups showed decreased total arm entries ($F_{(2,86)} = 19.376$, $p < 0.01$) in comparison to the controls (Figure 1d). No overall sex differences ($F_{(1,86)} = 1.519$, $p > .05$) or sex by treatment interactions ($F_{(2,86)} = 0.191$, $p > .05$) were seen.

3.2 IMO stressor and partner presence altered OTR receptor density in a brain region-specific manner

OTR autoradiographic binding was performed on the vole brain sections ($n=8$ per sex per treatment group) and the receptor densities were quantified in several brain areas known to play a role in social and/or anxiety-like behaviors (Figure 2). OTR binding density was expressed as a percent difference from the baseline controls. Significant group differences were found in the NAcc ($F_{(2,39)} = 6.641$, $p < 0.01$) (in both the core ($F_{(2,39)} = 6.086$, $p < 0.01$) and the shell ($F_{(2,39)} = 6.760$, $p < 0.01$)). Posthoc tests indicated that IMO-alone subjects showed significantly lower levels of OTR binding in the NAcc, compared to the controls (Figure 3). The level of OTR binding in the NAcc of the IMO-partner group did not differ significantly from either the control group or the IMO-alone group. Group differences in the OTR binding were only found in the NAcc and not in other examined brain areas including the LS, PFC, VMH, CeA, and BLA (Figure 3). No sex by treatment interactions were seen in any of the measured brain areas.

3.3 IMO stressor did not alter V1aR densities in measured brain areas

Along with OTR, V1aR autoradiographic binding was also performed on an alternate set of brain sections and V1aR densities were measured in selected brain areas (Figure 4). No significant group differences or sex by treatment interactions were found in any of the measured brain regions (Table 1).

3.4 Sex differences in OTR/V1aR densities were found in selected brain areas

Our data analyses also indicated overall sex differences in the density of OTR and V1aR binding in the brain. Male voles had significantly higher levels of OTR binding in the LS ($F_{(1,42)} = 4.520$, $p < 0.05$) and CeA ($F_{(1,38)} = 4.300$, $p < 0.05$) in comparison to females (Figure 5). No sex differences were found in OTR binding in other measured brain areas including the NAcc, PFC, VMH, and BLA. Female voles had a higher levels of V1aR binding in the MeA ($F_{(1,39)} = 11.057$, $p < 0.01$) compared to males (Table 2). No significant sex differences were found in the V1aR binding in other brain areas including the PFC, BNST, CeA, VMH, or LS (Table 2).

4. Discussion

Previous research demonstrates that social interactions with a bonded partner during the recovery period following a 1-hour IMO stress can induce “social buffering effects” in prairie voles by reducing their neurochemical, physiological, and behavioral stress responses that typically result from the IMO experience [20]. Through use of a novel paradigm in the current study, we have shown that the presence of a bonded partner during IMO stress did indeed prevent the typical rise in anxiety-like behaviors in both male and female prairie voles. In addition, this buffering effect on the stress response was associated with a brain region-specific alteration in OTR expression in the NAcc – these alterations were in a neurochemical-specific manner, as no effects were found on V1aR expression in any of the brain areas examined. Together, these data have not only revealed the NAcc as a new brain area in which OT may mediate social buffering effects on stress responses, but have also indicated a potential preventative approach in which enhancing social interactions with a partner during stress may negate detrimental outcomes that typically arise from the stressor.

Previous studies have shown that stressors such as immobilization, social isolation, social defeat, and partner loss can elicit physiological and behavioral stress responses in prairie voles [18, 47, 50–52]. Consistent with previous studies [20, 47], our data demonstrated that the IMO experience is stressful, as it significantly elevated anxiety-like behavior during an EPM test. Most interestingly, our data showed that the presence of a pair-bonded partner during the IMO stress reduced the anxiety-like behaviors elicited by the IMO experience, and such effects were behavior-specific, as it did not affect general locomotor activities. Previous prairie vole studies demonstrate similar effects of partner presence during stress recovery [19, 20]. Therefore, presence of pair-bonded partners during or after stressful experiences can have buffering effects in prairie voles by reducing their behavioral stress responses. This is consistent with the data from other traditional animal models. For example, marmosets placed into a novel cage with a social partner for 48 hours do not show a rise in urinary cortisol, which is usually induced by exposure to novel environments [53].

Rats accompanied by a partner display decreased fear withdrawal in comparison to those alone [54]. Rats can also show a social buffering effect on anxiety-like behaviors from social defeat stress. In this case, physical contact is necessary, as those housed with a cagemate separated by a wire mesh do not show similar buffering effects [55]. Interestingly, data from our present study showed that limited physical contact, along with olfactory and auditory cues, were sufficient to induce social buffering effects in prairie voles. These data may indicate the unique feature of male-female pair bonding and the potency of its associated auditory and olfactory cues in mediating neurochemical and behavioral adaptations to stress in species with monogamous life strategies [56–59].

Our behavioral data also show that male and female prairie voles had similar social buffering effects by their partners in response to the IMO stressor using the current paradigm. However, sex differences in social buffering have been reported in some rodent studies. For example, pair-housing in rats after a foot-shock stressor alters anxiety-like behaviors in a sex-dependent manner [60], while social support also alters stress behaviors differently between male and female guinea pigs [61]. It is worth mentioning that while sex differences are found in social bonding and its underlying neurochemical mechanisms [12, 16], disruption of social bonds [62] or experience with a stressful event such as foot-shock [19] can induce similar neurochemical and behavioral responses in both male and female prairie voles. While species differences may account for the divergent findings among studies, the lack of sex differences in response to IMO stress in prairie voles may provide additional evidence to further support the notion that comparable outcomes may allow males and females to similarly adapt to environmental challenges, particularly in species that adapt monogamous life strategies and require cooperative breeding [63].

The region-specific OTR alterations seen in the NAcc pose a new, interesting role of OT in the reward circuit in response to acute stressors. The OT system has been well documented to modulate stress coping in a variety of animal models, and it can alter typical deficits seen from chronic restraint stress as well [64, 65]. The NAcc is well known to be a critical region for various rewarding behaviors [66–68] including the pair-bonding behavior seen in prairie voles [69–71]. In a most recent study in male prairie voles, NAcc-specific alterations to the OT system have been shown to mediate stress-coping behaviors associated with the loss of a pair-bonded partner [51]. Our data showed that increased anxiety-like behavior by the IMO stressor was associated with a decrease in OTR binding in the NAcc, and this effect was dampened by the partner's presence. As OT in the brain can buffer neurochemical and behavioral responses to a variety of stressors in general [10, 28, 64, 72], and to IMO stressors in prairie voles in particular [20], our data suggest that decreased OTR signaling in the NAcc may be involved in stress responses. Further, NAcc OTR may also be involved in mediating social buffering effects on stress responses elicited by the partner-associated stimuli in prairie voles. It is worth mentioning that both the IMO stressor and the bonded partner facilitate OT release in the PVN of the hypothalamus in female prairie voles [20]. It would be interesting to see if similar effects also exist in the NAcc. Nevertheless, the data collectively indicate that NAcc OT plays an important role in pair bond formation while also having a functional significance of enhancing the likelihood of success in the monogamous life strategy [51].

The brain AVP system is known to be involved in stress responses as well as in social behaviors [31, 73]. For example, V1aR in the brain is altered by social defeat experience in California mice [74] and is involved in modulating behavioral reactions to a threat in rats [75]. V1aR in selected brain areas is also implicated in a variety of social behaviors including social affiliation and recognition [40, 41], parental behavior [63, 76], and pair bonding [37, 77]. Surprisingly, our data indicated no group differences in the density of V1aR binding in any of the brain areas examined in prairie voles. Although these data can be interpreted as no involvement of the brain AVP system in IMO stress and social buffering on such stress responses, previous studies have indeed shown that V1aR is involved in stress responses from various types of stressors in other species [78–82], yet no stress response effects have been examined in the V1aR system in prairie voles. It is possible that several factors, such as the type [83] and/or intensity [78] of the stressor, might have attributed to the lack of treatment effects on V1aR binding in the vole brain in the present study. Additional experiments will need to be conducted to continue to examine the role of the brain AVP system in social buffering of stress responses.

Another interesting set of data from the present study is the sexually dimorphic patterns of OTR and V1aR in a brain region-specific manner in prairie voles. Sex differences in OTR and V1aR in the brain have been reported for many traditional laboratory animals [84, 85], along with different physiological and behavioral functions of OT and AVP between males and females [86]. In prairie voles, sexual dimorphisms are found in the distribution patterns of OTR and V1aR [34, 35] and, to a less extent, in the density of OT and AVP producing cells [87, 88] in the brain. Collectively, behavioral data suggest that AVP is more important in males, whereas OT is more important in females in the regulation of their social behaviors [14, 37, 89]. To our knowledge, the present study is the first to report a higher level of V1aR binding in the MeA in females in comparison to males, as well as higher levels of OTR binding in the CeA and LS in males than in females. In general, previous data indicate a male-biased AVP system and a female-biased OT system [89] – thus, such reversed patterns indicated by our data is interesting and worth future investigation. The functional significance of such sexual dimorphisms in OTR and V1aR is still unclear, and therefore, we can only speculate. In the LS, for example, males have a higher density of AVP immunoreactive projections from the BNST, compared to females [87], and locally released AVP plays an important role in male pair bonding and paternal care behavior [76, 77]. As concurrent access to V1aR and OTR in the LS is required for AVP regulation of pair bonding behavior in male prairie voles [77], high OTR expression in the LS of male voles may facilitate AVP regulation of pair bonding. This may be true, as data from recent studies suggest interactions between AVP and OT systems in the brain [90, 91]. Nevertheless, this speculation needs to be tested in subsequent experiments. Given the intricate roles of OT and AVP in social behaviors, these sexually dimorphic results could provide a new direction for more research on microcircuit receptor differences that may underlie sex differences in behaviors.

In summary, data from the present study indicated that the presence of a bonded partner during an IMO stress could have social buffering effects on anxiety-like behaviors in both male and female prairie voles. In addition, experience in the IMO stressor, as well as with the partner-induced social buffering, is associated with alterations in OTR in the NAcc.

These data further demonstrated the potential involvement of complex neural circuitry of neuropeptides (e.g., OT and AVP) in the regulation of stress responses and social buffering of stress responses [19, 20, 73, 78, 83]. It should be mentioned that because a bonded partner was used as the social stimuli in our study, we have described the effects as “from the partner”. However, additional groups of subjects that undergo the IMO stressor in the presence of a novel, same-, or opposite-sex conspecific should be included in further experiments to reveal the potential specificity of the social stimuli on buffering stress responses. Furthermore, as previous studies have demonstrated that IMO experience can induce hormonal stress responses in prairie voles [20, 47], we expect that the IMO stress also increased circulating levels of corticosterone in the present study. Although a partner’s presence during the recovery period following the IMO stress was effective at buffering corticosterone responses in female prairie voles [20], we still don’t know if partner’s presence during the IMO stress can reduce circulating corticosterone. This caveat needs to be addressed in further studies, as the partner may have similar or differential buffering effects on hormonal and behavioral stress responses in a stage-specific manner (e.g., during or after the IMO stress). In addition, the combination of hormonal and neurochemical data (e.g., OTR) may indicate similar or differential roles of the two systems on social buffering of behavioral stress responses. Finally, the translational value of our data should also be noted. In humans, the physical presence of a close partner or friend can diminish cortisol concentrations, and social support during a social stress test can reduce perceived anxiety during the stressor [72]. As social environments are a critical part of our lives, we must continue to explore this area of research to understand how social bonds may ultimately shape our health outcomes and wellbeing. The availability of the prairie vole model provides an excellent opportunity to continue to study the neural circuitry and neurochemical mechanisms underlying stress and social buffering.

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Highlights

- IMO stress increases anxiety-like behavior
- Partner presence during IMO stress buffers typical rises in anxiety-like behavior
- IMO stress decreases OTR expression in the NAcc in a brain region-specific manner
- Sex differences are found in OTR and V1aR expression in the brain of prairie voles

Figure 1. Partner presence during IMO stress reduced anxiety-like behavior

No differences were found between IMO-stressed prairie voles and non-stressed controls in the latency to enter the open arms of the EPM (A). IMO stress decreased total number of open arm entries in both IMO stress groups in comparison to non-stressed controls (B). Prairie voles that received IMO stress alone spent significantly less time in the open arms of the EPM in comparison to those that received IMO stress with pair-bonded partners and non-stressed controls (C). Both IMO stress groups showed similar locomotor activity, with a decreased total number of arm entries in comparison to the non-stressed control group (D). EPM, elevated plus maze, IMO, immobilization stress. As no sex by treatment interactions were found, the data were pooled from males and females. Bars indicate mean $\pm$ SEM. Bars with different letters differ significantly from each other.

Figure 2. Autoradiographic images of OTR binding in selected brain regions

Autoradiographic images of OTR binding in the NAcc (A), LS (B), PFC (C), and AMY (D) in non-stressed control voles (Control), voles that received IMO alone (IMO-Alone), and voles that received IMO stress with the presence of their partners (IMO-Partner). Groups included both male and female subjects. AC, anterior commissure, BLA, basolateral amygdala, CC, corpus callosum, CeA, central amygdala, IMO, immobilization stressor, LV, lateral ventricle, LS, lateral septum, NAcc, nucleus accumbens, OT, optic tract, PFC prefrontal cortex. Scale bar = 500 μ m.

Figure 3. IMO stressor altered OTR density in a brain-region specific manner

OTR binding density in the NAcc in the IMO-Alone group was significantly lower in comparison to non-stressed (Control) voles. The level of OTR density in the NAcc in voles which received IMO with partners (IMO-Partner) did not significantly differ from the other two groups. No differences in OTR density were found in other brain regions measured including the LS, PFC, VMH, CeA, and BLA. As no sex by treatment interactions were found, the data were pooled from males and females. BLA, basolateral amygdala, CeA, central amygdala, IMO, immobilization stressor, LS, lateral septum, NAcc, nucleus accumbens, OTR, oxytocin receptor, PFC, prefrontal cortex, VMH, ventromedial hypothalamus. Bars with different letters differ significantly from each other.

Figure 4. Autoradiographic images of V1aR binding

Autoradiographic images of V1aR binding in various brain regions. AC, anterior commissure, ACo, cortical amygdala, BNSTd, dorsal bed nucleus of stria terminalis, BNSTv, ventral bed nucleus of stria terminalis, CC, corpus callosum, CeA, central amygdala, LS, lateral septum, MeA, medial amygdala, OT, optic tract, PFC, prefrontal cortex, VMH, ventromedial hypothalamus. Scale bar = 500 μ m.

Figure 5. Sex differences in OTR density were found in selected brain areas

Overall sex differences were found in OTR autoradiographic binding. Male prairie voles had significantly higher OTR binding in the LS and CeA in comparison to female prairie voles. No sex differences were found in the NAcc, PFC, VMH, or BLA. As no sex by treatment interactions were found, the data were pooled from all treatment groups. BLA, basolateral amygdala, CeA, central amygdala, LS, lateral septum, LV, lateral ventricle, MeA, medial amygdala, NAcc, nucleus accumbens, OTR, oxytocin receptor, PFC, prefrontal cortex, VMH, ventromedial hypothalamus. Scale bar = 500 μ m.

Table 1

Group differences in the densities of V1aR binding in the brain of prairie voles

Brain Region	Control	IMO-alone	IMO-partner	F	p
PFC	44.19 ± 4.60 *	40.32 ± 5.11	36.50 ± 4.73	0.679	ns
BNSTd	54.19 ± 2.78	58.94 ± 2.88	58.37 ± 3.00	0.843	ns
BNSTv	94.27 ± 2.36	90.10 ± 2.36	93.89 ± 2.46	0.944	ns
CeA	81.29 ± 2.38	77.29 ± 2.47	78.64 ± 2.47	0.710	ns
MeA	78.31 ± 2.27	82.07 ± 2.43	79.37 ± 2.33	0.671	ns
CoA	49.74 ± 3.17	49.85 ± 3.26	50.53 ± 3.26	0.018	ns
VMH	75.49 ± 4.15	85.27 ± 4.75	87.98 ± 4.89	2.213	ns
LS	83.53 ± 6.69	88.19 ± 7.13	91.66 ± 7.13	0.351	ns

* Mean ± SEM

Table 2

Sex difference in the densities of V1aR binding in the brain of prairie voles

Brain Region	Male	Female	F	p
PFC	42.69 ± 3.89 *	37.98 ± 4.01	0.713	ns
BNSTd	57.34 ± 2.39	56.99 ± 2.32	0.011	ns
BNSTv	92.94 ± 1.96	92.57 ± 1.95	0.017	ns
CeA	76.48 ± 1.95	81.67 ± 2.04	3.391	ns
MeA	75.42 ± 1.88	84.41 ± 1.94	11.057	.002
CoA	50.95 ± 2.63	49.12 ± 2.64	0.241	ns
VMH	83.51 ± 3.72	82.32 ± 3.80	0.050	ns
LS	86.44 ± 5.75	89.15 ± 5.65	0.112	ns

* Mean ± SEM