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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-h 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.

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

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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-h 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 lx. 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 min 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 min before beginning the receptor binding processing. Brain sections were washed twice, each for 10 min, 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 ^{125}I-LVA for V1aR or (PerkinElmer NEX 310) 50 pM ^{125}I-OVTA for OTR (PerkinElmer NEX 254) for 90 min at room temperature. After the 90-min 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 min, and then washed in chilled Tris-HCL with 10 mM MgCl2 four times for 5 min each. Sections were then placed in chilled Tris-HCL with 10 mM MgCl2 for 30 min with stirring. After the 30-minute stirring, all sections were dipped in ddH20 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