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Effect of Partner Separation on PVN Vasopressin Cells and Behavior in Mongolian Gerbils.

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Effect of Partner Separation on PVN Vasopressin Cells and Behavior in Mongolian Gerbils.

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Introduction: Plasticity of the paraventricular nucleus of the hypothalamus (PVN) vasopressin (VP) cell group has previously been demonstrated in prairie voles, such that cell numbers are different based on pair-bonding status. Whether this bond status-dependent plasticity occurs in other socially monogamous species is unknown. Here we explored the effect of partner separation on a spectrum of behaviors as well as on vasopressin anatomy and function in the PVN.

Methods: Sixteen male Mongolian gerbils were randomly assigned to either a Paired or Separated group and were allowed to cohabitate with an opposite-sex partner for two weeks. After cohabitation, subjects were run through a set of behavior tests, and were either separated from their partner (Separated group) or remained with their partner (Paired) for four weeks, and tested again through the same set of behavior tests. Lastly, an immediate early gene (IEG) social interaction test was run before brains were collected for immunohistochemical staining of VP-ir and Fos-ir cells to visualize neural activity of interest.

Results: In a reproductive context, separated male Mongolian gerbils showed increased time spent investigating and acting prosocially toward an opposite-sex conspecific. In a non-reproductive social context, males did not show behavior changes after they were separated from their partner. Within a non-social context, separated males traveled a lesser amount of distance compared to paired males. Separated males had higher PVN VP-ir cell counts, which positively correlated with the amount of time spent investigating an opposite-sex conspecific, compared to paired males.

Conclusion: Our results suggest that partner separation does not generally alter behavior in non-reproductive contexts, such as non-social anxiety-like behavior and interaction toward same-sex conspecifics. However, subjects that were separated from their partners exhibited more prosocial and investigative behaviors in a reproductive context, and the investigative behavior positively correlated with VP cell densities in the PVN. We also found that separated males had higher VP densities in the PVN than males who remained paired, suggesting that there is an increase in PVN VP density following separation from the partner, possibly to increase investigation of opposite-sex conspecifics to find a new mate and/or to alleviate stress.

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INTRODUCTION

Pair-bonding is a psychological construct describing a lasting, exclusive relationship between two organisms that often include partner preference, territoriality/mate guarding, separation distress, and stress buffering (Bales et al., 2021). In mammals, researchers often identify pair-bonding by an enduring display of partner specific affiliative behaviors extending beyond one reproduction cycle and selective aggression toward unfamiliar conspecifics of the opposite sex (Kleiman 1977; Young et al. 2008). With the benefit of self-reported affect, pair-bonding in humans takes the form of romantic relationships and love. The loss of a partner can result in negative consequences on mental and/or physical health. Thus, neurobiological research on pair-bonding can provide translational insight into how we form and cope with the loss of bonds with our special conspecifics. Mongolian gerbils (*Meriones unguiculatus*) are an excellent organism for studying how the brain modulates pair-bonding-related behaviors. These diurnal rodents dwell in burrows in small family groups and are native to desert grasslands of China, Mongolia, and Russia (Liu et al., 2009). They are socially monogamous, biparental, and exhibit aggression toward unfamiliar conspecifics of the same sex, making them a good model organism to explore prosocial behavior with mates and selective aggression toward novel conspecifics. (Hendrie and Starkey, 1998; Pina-Andrade et al., 2020; Kelly et al., 2022). Although studies have examined pair-bonding behavior in gerbils, much less is known about how the brain modulates pair-bonding in this species (Taylor et al., 2023). Across taxa, neurobiological research on pair-bonding has converged on examination of the nonapeptide system, including vasopressin (VP) and its respective homologues (Cardoso et al., 2015; Freeman & Young, 2016; Walum & Young, 2018). In rodents specifically, VP is involved in regulating pair-bonding, affiliation, and aggression-related behaviors. In the extensively studied socially monogamous prairie vole, VP

modulates post-mating partner preference formation (Winslow et al., 1993); for male voles specifically, VP activation of V1a receptors (V1aR) is required in addition to oxytocin (OT) receptor activation to form and express a mating-induced partner preference (Donaldson et al., 2010; Winslow et al., 1993). Importantly, there is also evidence that central VP systems may be important for pair bonding in female prairie voles (Cho et al., 1999). Among closely related species, however, drastic variations in pair-bonding capabilities have been observed. For example, cross-species comparisons of partner preference tests in Mongolian gerbils, midday jirds (*M. meridianus*), and pale gerbils (*Gerbillus perpallidus*) highlighted Mongolian gerbils' high sociability and robust partner preference, in contrast to a lack of selective aggression from nonterritorial *M. meridianus* and a preference to stay solitude from territorial promiscuous *G. perpallidus* (Tchabovsky et al., 2018). Similarly, the numbers of VP cells in hypothalamic brain regions were greater in the Mongolian gerbil than in the Great gerbil and Midday gerbil (Yu et al., 2020); differences in the arrangement of nonapeptide receptors have been found to influence social behavior both within and between species, as demonstrated by comparisons of prairie voles with their nonmonogamous relatives, the meadow and montane voles (Sadino and Donaldson, 2018). Notably, delivering prairie vole V1aR gene to promiscuous meadow voles by viral vector significantly enhanced partner preference, highlighting VP's modulatory capability (Lim et al., 2004). Still, the underlying neural mechanism for rodent pair-bonding is not thoroughly understood; collectively, evidence from prior literature suggests VP signaling regulates pair-bonding related social behavior in a species-specific way. Thus, the translational power of pair-bond research could benefit from studying species other than prairie voles, such as the Mongolian gerbil. Recent studies from prairie vole research has shed light on the consequences of partner separation. For example, at least males are versatile enough to go

through formation and dissolution of a pair-bond up to 10 times (Kenkel et al., 2019). Consistent with this, partner separation leads to PVN OT rebounds after initial neural densities decrease following pair-bonding (Fricker et al., 2023), suggesting that the ability to repeatedly form pair-bonds may be possible due to robust neural plasticity in pair-bond related circuits. Whether nonapeptide neuronal populations exhibit such plasticity in relation to pair-bonding in species other than prairie voles remains unknown. Further, although dissolution of a pair-bond influences nonapeptide neuroanatomy, it is currently unknown whether it influences nonapeptide function. Here I propose to compare the brain and behavior of male Mongolian gerbils that were either in a stable pair-bond or had been recently separated from their pair-bond partner. To examine how pair-bonding and/or partner separation may influence behavior related to as well as not related to pair-bonding (i.e., exploratory behavior, interactions with novel conspecifics, etc.), I used a within-subjects design and tested males at two timepoints in a battery of behavioral tests. After behavioral testing, I conducted an immediate early gene study to determine whether VP neurons exhibit differential neural activity in response to an interaction with a novel female in a manner dependent on pair-bonding status. Changes in VP cell densities will be examined in the paraventricular nucleus of the hypothalamus (PVN). The PVN contains one of the most concentrated VP cell populations in the brain, with dynamic projections to the pituitary and throughout the brain. We recently showed that the PVN VP cell group exhibited neuroanatomical plasticity in relation to pair-bonding status in prairie voles (Fricker et al., 2023). We predict that this dynamic brain region evolved to be flexible across species, and that we will observe differences in neural densities and/or function in relation to pair-bonding status in the gerbils.

MATERIALS AND METHODS

Animals

Sixteen adult male *M. unguiculatus* (post-natal day (PND) 60–200) were used for all behavioral tests in this experiment. All Mongolian gerbils were obtained as young adults (postnatal day (PND) 50–65) from Charles River Laboratories and tested between PND 80–100. Gerbils become sexually mature by PND 60.

Gerbils were group housed (2–4) with same-sex littermates in standard rat polycarbonate cages (40.64 × 20.32 × 20.32 cm) prior to the establishment of male-female pairs. All cages were lined with Sani-Chips bedding and included nesting material, chewing blocks and shepherd shacks. Animals were able to obtain food and water ad libitum and were kept on a 14 L : 10 D cycle. Ambient temperatures were maintained at 24 ± 2°C. All procedures were approved by the Institutional Animal Care and Use Committee of Emory University (PROTO202100074 & PROTO201900126).

Experimental Design

Male subjects (n=16) were randomly assigned to one of the two groups: (1) Pair-bonded or (2) Partner Separation. All subjects were paired with an adult female and allowed to cohabitate for 12 days prior to the beginning of testing; 48 hrs of cohabitation is sufficient for male to form partner preference (Tchabovsky et al., 2019). Sex was defined based on external genitalia. Only males were used as subjects. This unisexual focus of testing and tissue harvest is to reduce animal usage and make a more comparable analysis of results from existing literature that focuses on males (Winslow et al. 1993; Lim et al. 2004; Ophir et al. 2008; Solomon et al. 2009;

Mabry et al. 2011; Blocker and Ophir 2016), since sexual conflict theory predicts that males are more likely to desert their partners than females (Trivers 1972).

After 12 days of cohabitation with a pair-bond partner, male subjects underwent a set of behavioral tests in a randomized order over 3 days. After this first set of behavioral testing (henceforth referred to as Timepoint 1), subjects in the Partner Separation group had their pair-bond partner removed and they were single-housed for 4 weeks. Subjects in the Pair-bonded group remained co-housed with their partner. After 4 weeks, all subjects underwent a second round of the same set of behavioral tests (referred to as Timepoint 2). The battery of behavioral tests (at Timepoint 1 and Timepoint 2) included: social interaction test, social approach test, resident intruder test, open field test, partner preference test, social effort test. Testing sequence was randomly assigned to discount systematic influence of one test to the outcome of subsequent tests. All stimulus animals were novel for the subjects in each behavioral test. After the completion of testing at Timepoint 2, subjects underwent an immediate early gene test in which they were exposed to a novel female conspecific for 30 minutes before perfusion.

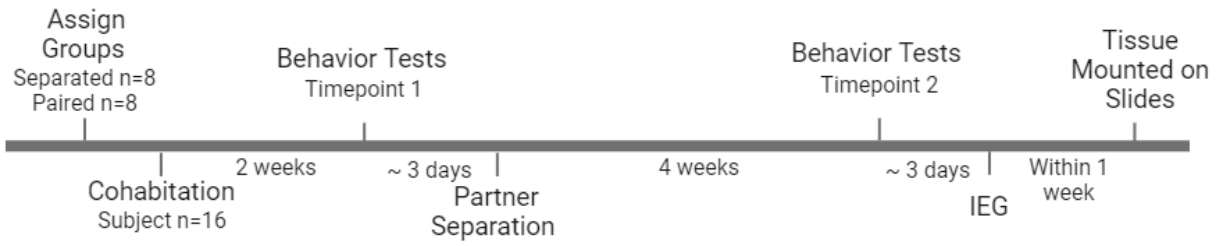

Figure 1: General Experimental Timeline

Behavioral Tests

Social Approach Test

To assess whether motivation to engage socially with a potential mate was differentially modulated by bonding status (paired or separated), subjects' latency to approach an opposite-sex conspecific was measured. Social Approach is tested in a large Plexiglas testing chamber (81.28 × 40.64 × 38.1 cm). Prior to testing, subjects were allowed to freely explore the empty chamber for 3 minutes. After acclimation, subjects were contained under a plastic beaker on one end of the chamber, while a stimulus animal was placed under a wire mesh container on the contrary end of the chamber. The subjects were then released and the latency to approach was measured. Operationally this means the duration between subjects' release and when they made physical contact with the wire mesh container.

Behavior	**Description**
aggressive side-by-side contact	Subject and stimulus are touching flanks but in an aggressive manner. Maybe between aggression bouts.
Allogrooming	Subject grooms the stimulus animal
Autogrooming	Subject grooms itself.
Biting	Subject bites at the stimulus animal, mouth making contact with the stimulus animal's body.
Chasing	Subject is aggressively chasing the stimulus animal. Initiator is the chaser for the entire event.
Huddling	Subject and stimulus are either touching flanks or criss-crossed on top of each other.
Lunging/attacks/rolling	Subject lunges forward, swipes, or rolling around paws at the stimulus vole in

Behavior	Description
	aggression.
Pinning (Aggression)	Pinning the stimulus down
head investigation	Subject sniffs or positively investigates the head or neck region of the stimulus animal
Flank Investigation	Subject sniffs or positively investigates the flank of the stimulus animal
Rear Investigation	Subject sniffs or positively investigates the rear of the stimulus animal

Table 1: Ethogram for Behavioral Scoring in Social Interaction and Resident Intruder Tests.

Resident Intruder Test

To assess how bonding status affects subjects' social behavior toward a potential mate, subjects were allowed to freely interact with a novel opposite-sex stimulus in subjects' home cages for 10 minutes (40.64 × 20.32 × 20.32 cm). There was no acclimation time for this test since the subjects are already familiar with the environment. Behaviors scored (Table 1) included the duration of subject's investigation behavior (head investigation, flank investigation, and rear investigation), prosocial behavior (huddling, allogrooming), aggressive behavior (pinning, lunging/attacking, aggressive side-by-side contact, biting, chasing) and non-overt behaviors (all other behaviors not considered overtly prosocial or aggressive, including autogrooming, jumping, sitting alone, and nonsocial exploration).

Social Interaction Test

To assess the effect of partner separation on Mongolian gerbils' social behavior in a non-reproductive context, subjects were allowed to freely interact with a novel same-sex

stimulus animal for 10 minutes. Both subjects and stimulus animals were released into a novel testing chamber (40.64 × 20.32 × 20.32 cm) at the same time, after which 10 mins of interaction were recorded and scored. Behaviors scored were the same as in the RI test (Table 1).

Open Field Test

To assess potential changes in anxiety and general locomotion, subjects were allowed to freely explore a 120 x 120 cm square open field for 10 minutes. Subjects were released from the center of the open field, and their velocity, distance traveled and the time in the central and fringe area over 10 minutes were automatically measured through Ethovision XT (Noldus, Information Technology, The Netherlands). The arena was subdivided into a center region (38.4 x 38.4 cm), a buffering zone, and a border region (24cm x 50 cm on four sides) in Ethovision, similar to previous studies using open field tests (Wang et al., 2017).

Immediate-early gene experimental design

To correlate potential behavioral changes with neuron population densities, subjects (n=16) underwent a social interaction test for 30 min before they were perfused for subsequent immunofluorescent visualization of VP and the immediate early gene Fos. Subjects were placed in a new standard rat cage and were allowed to acclimate for 20 minutes. Then, an opposite-sex conspecific was placed into the testing chamber for 30 minutes, after which it was removed. The subject remained for another 60 minutes and was immediately perfused. Behavior (Table 1) was scored for the first 10 min of the social interaction as this period likely most closely corresponds to the immediate-early gene responses quantified.

Histology and Immunohistochemistry

Perfusion and fixation

Subjects were euthanized by isoflurane overdose, and transcardial perfusions were performed with 0.1 M PBS followed by 4% paraformaldehyde dissolved in 0.1 M borate buffer (pH 9.5). Extracted brains went through post-fixation in 4%paraformaldehyde dissolved in 0.1 M borate buffer (pH 9.5) overnight, before cryoprotected in PBS with 30% sucrose for 48 h. Subsequently, brains were frozen in TissueTek O.C.T. Compound (Sakura Finetek) in Peel-A-Way molds and stored at -80°C.

Sectioning

Prior to immunohistochemical processing, brains were thawed and sectioned coronally at 40um using a Leica cryostat, with one in every three sections retained for this study. Sliced tissue were stored in 1.5 ml VWR® microcentrifuge tubes filled with cryoprotectant at -80°C. Afterwards, selected hypothalamic sections were transferred to 0.1M PBS (pH 7.4) on well plates, and were immunofluorescently stained for VP and Fos.

Immunohistochemistry procedure

Selected tissues underwent block, primary, biotin, and secondary steps. All incubation took place in a humid chamber lined with napkins soaked by deionized water. Diluent for all antibodies and biotin contained 0.1M PBS with 5% normal donkey serum and 0.03% Triton X-100. Specific procedures are as follows: sectioned tissues were rinsed 3x in 0.1 M PBS for 10 minutes on the shaker. Then, tissues were transferred into a blocking solution (10% normal donkey serum, 0.3% triton, PBS), and incubated at room temperature for 1 hr. Subsequently, tissues were transferred

into the primary solution and stored at 4°C for 48 hours. After incubation in primary antibodies, tissues were rinsed in 0.1M PBS for 30 minutes on the shaker twice. To increase the number of enzyme molecules bound to tissue, we bathe sections in biotin solutions (8:1000 donkey anti-guinea pig biotin). Following two rinses, tissues were transferred into secondary antibodies, and were incubated at room temperature in the dark for two hrs. Following two more 20 min rinses in PBS, tissues were stored in PBS on well plates inside a humid chamber at 4°C. Sections were mounted on microscope slides, and cover-slipped with Prolong Gold antifade containing a DAPI nuclear stain (ThermoFisher Scientific). All stored tissues were mounted within one week after incubation in secondary antibodies.

Antibody Composition

Primary antibodies: guinea pig anti-VP (1:1000; PenLabs) and rabbit anti-Fos (1:1000; SySy); Secondary antibodies: donkey anti-mouse secondary conjugated to Alexa Fluor 488 (3:1000), donkey anti-rabbit secondary conjugated to Alexa Fluor 594 (3:1000).

Data Acquisition and Analysis

All behavioral tests were video recorded using Sony Handycam HDR-CX405 1080p Camcorders (Sony). Open Field Tests were auto-processed using EthoVision XT; all other behaviors were manually quantified with Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). PVN VP cell density and its colocalization with c-Fos was quantified with Fiji (ImageJ; Schindelin et al., 2012). All statistical analysis was performed in IBM SPSS Statistics (Version 29). Repeated Measures General Linear Models (rmGLMs) were used to analyze behavioral data and Mann Whitney U-Tests were used to analyze brain data. All post hoc

pairwise comparisons were adjusted using the Sidak correction. Graphs were made using Prism 8 (GraphPad, USA).

RESULTS

Effects of partner separation on nonreproductive behaviors

We first examined whether 4 weeks of separation from a pair bond partner influenced boldness in an open field test. A rmGLM with Pair Bond Status (Paired or Separated) as a fixed factor and Time (Timepoint 1 vs. Timepoint 2) as a repeated measure revealed no effects or interactions, such that males that were pair bonded did not exhibit differences in time spent along the periphery (all $p > 0.57$) or in the center (all $p > 0.98$) of the open field chamber compared to males that were separated. However, we found a main effect of Pair Bond Status on the distance moved during the open field test ($p < 0.01$; $F_{(1,13)} = 10.48$), showing that pair bonded males moved more than separated males.

We next examined whether partner separation influenced social behavior during interactions in a nonreproductive context (i.e., with a novel, same-sex conspecific). A rmGLM with Pair Bond Status as a fixed factor and Time as a repeated measure revealed no effects or interactions for investigation (all $p > 0.53$), prosocial behavior ($p > 0.59$), non-overt behavior ($p > 0.61$), or aggressive behavior ($p = 0.065$). Because we observed a marginal trend for aggression, we conducted exploratory post hoc analyses, which showed that within pair bonded males, there is

an increase in aggression from Timepoint 1 to Timepoint 2 ($p = 0.04$; MD = 38.77; Figure 2). This suggests that pair bonded males may become more aggressive in a nonreproductive context the longer they have been pair bonded.

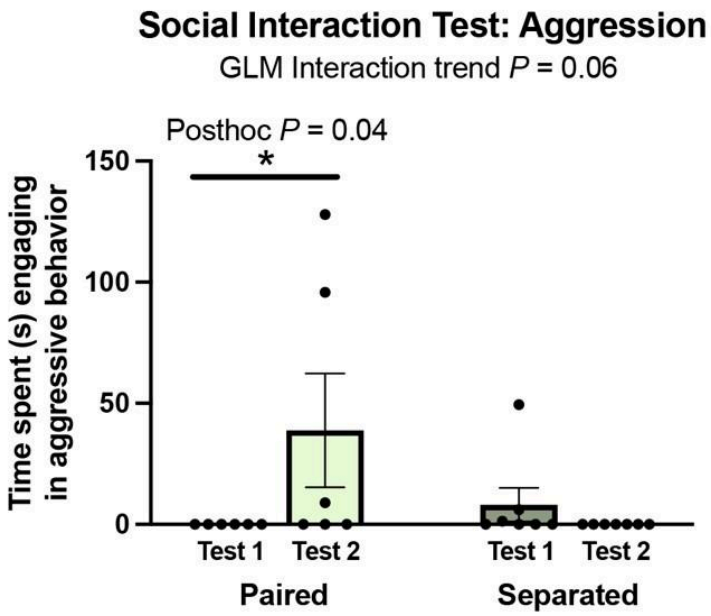

Figure 2: Pair bonded males exhibited more aggression in the social interaction test at Timepoint 2, after a total of 6 weeks of being pair bonded. Separated males did not show a change in aggression toward a novel, opposite-sex conspecific between the time they were pair bonded (Test 1) and after they had been separated from their partner for 4 weeks (Test 2).

Effects of partner separation on reproductive social behavior

To determine if partner separation influenced social behavior in a reproductive context, male subjects were testing in a social approach test with a novel, opposite-sex conspecific and in a resident-intruder test with a novel, opposite sex conspecific in the subject's homecage. For the social approach test, a rmGLM with Pair Bond Status as a fixed factor and Time as a repeated

measure revealed no effects or interactions for the latency to approach the stimulus animal (all $p > 0.94$). Similarly, during the resident-intruder test, a rmGLM revealed no effects or interactions for prosocial behavior (all $p > 0.39$), non-overt behavior (all $p > 0.06$), or aggression (all $p > 0.07$). However, for investigation during the resident-intruder test, while we observed no effect of Pair Bond Status on time spent investigating the novel, opposite-sex intruder ($p = 0.42$), we found a significant interaction ($p < 0.01$; $F_{(1,13)} = 9.65$) between Pair Bond Status and Time. Sidak-corrected posthoc analyses showed that paired and separated males did not exhibit differences in investigation at Timepoint 1 or Timepoint 2 (all $p > 0.10$), but that within separated males, investigation increased from Timepoint 1 to Timepoint 2 ($p = 0.02$; MD = 54.07; Figure 3). This suggests that separated males may be more receptive to a potential new mate after 4 weeks of separation from their prior pair bond partner.

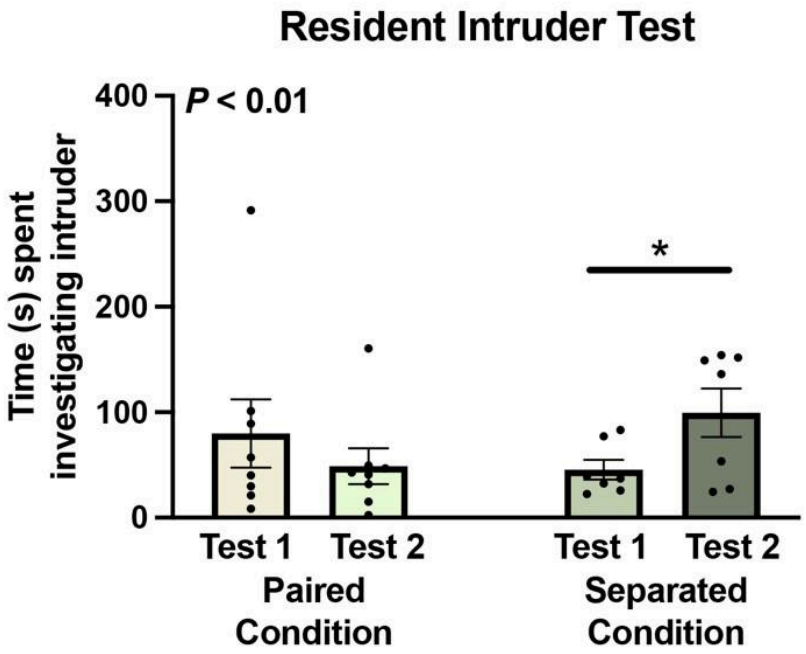

Figure 3: Investigation of a novel, opposite-sex intruder increased between the time they were pair bonded (Test 1) and after they had been separated from their partner for 4 weeks (Test 2). Behavior did not differ from Test 1 to Test 2 for paired males.

We also examined interactions with a novel, opposite-sex conspecific on neutral territory in the final IEG test (i.e., only a single test and not repeated measures). A Mann Whitney-U test revealed a main effect of Pair Bond Status on prosocial behavior ($p = 0.02$; $Z = -2.38$; Figure 4) and investigation ($p = 0.01$; $Z = -2.45$; Figure 5) of the novel, opposite-sex conspecific, showing that separated males were more prosocial and investigative of the novel female compared to paired males. There were no differences in aggressive or non-overt behavior in the social interaction IEG test (all $p > 0.16$).

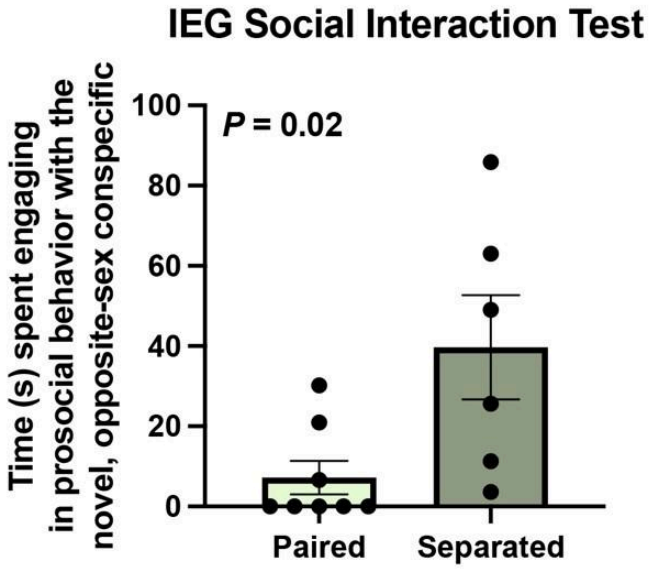

Figure 4: Separated males spent more time engaging in prosocial contact with a novel, opposite-sex conspecific on neutral territory compared to paired males.

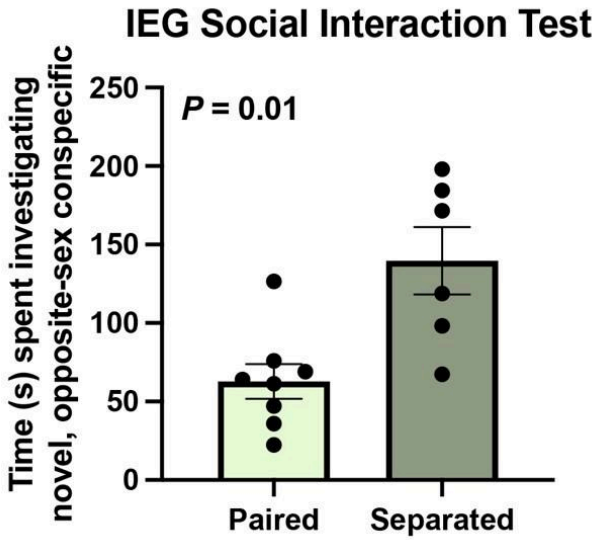

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Figure 5: Separated males spent more time investigating a novel, opposite-sex conspecific on neutral territory compared to paired males.

Effects of partner separation on PVN VP function

To determine if 4 weeks of partner separation influenced hypothalamic nonapeptide function, we examined PVN VP colocalization with Fos in response to a social interaction with a novel, opposite-sex conspecific on neutral territory. A Mann-Whitney U test yielded no difference in PVN VP-Fos colocalization between paired and separated males ($p = 0.12$; $Z = -1.55$; Figure 6a). This suggests that the responsiveness of PVN VP to a novel, opposite-sex conspecific is not significantly influenced by partner separation and/or pair bond status.

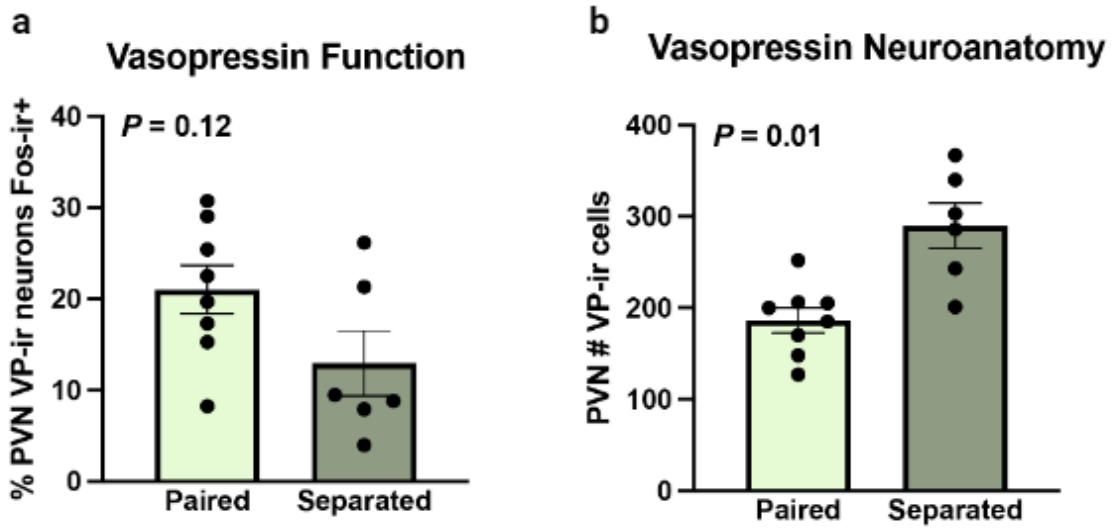

Figure 6: PVN VP density, but not PVN VP-Fos colocalization, differ between paired and separated males.

Effects of partner separation on PVN VP neuroanatomy

To determine if 4 weeks of partner separation influenced hypothalamic nonapeptide neuroanatomy, we examined VP neuronal densities in the PVN. A Mann-Whitney U test showed that males that were separated from their partners had significantly greater PVN VP cell densities compared to males that remained pair bonded with their partner ($p < 0.01$; $Z = -2.58$; Figure 6b). Because the social interaction IEG test was conducted as the final test immediately prior to perfusion of subjects, we next related PVN VP neuroanatomy to behavior during the social interaction with a novel, opposite-sex conspecific. PVN VP neural densities did not correlate to prosocial, non-overt, or aggressive behavior (all $p > 0.11$). However, we did find a significant correlation between PVN VP cell number and investigation ($p = 0.04$; Pearson's $R = 0.55$; Figure

7). This suggests that, regardless of pair bond status, PVN VP may promote social investigative behavior.

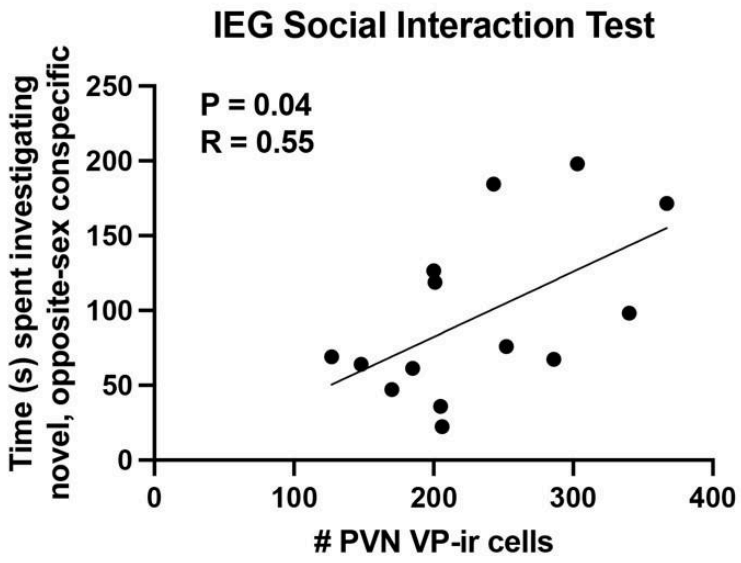

Figure 7: The total number of PVN VP neurons positively correlated with the time spent investigating a novel, opposite-sex conspecific on neutral territory.

DISCUSSION

Behavioral consequences of pair bonding

The overwhelming majority of studies examining pair-bonding have been conducted in prairie voles (Sadino & Donaldson, 2018;Blumenthal & Young, 2023). Early literature exploring behavioral consequences of pair-bonding in prairie voles concluded that 24 hrs of cohabitation is sufficient for male prairie voles to display both selective affiliation to their respective partners and selective aggression toward unfamiliar same sex conspecifics (Insel et al., 1995), two defining features of a pair-bond (Kleiman 1977; Young 2008). Another study later found bonded

males also display heightened aggression toward unfamiliar female conspecifics in a resident-intruder setting(Gobrogge et al., 2007), suggesting that, once bonded, males will reject potential mates in addition to exhibiting mate guarding. Such heightened aggression is also observed in pregnant or lactating female voles to unfamiliar males in laboratory settings (Thomas and Birney, 1979; Getz and Carter, 1980; Getz et al., 1981; Witt et al., 1990; Maestripieri, 1992).

Similar to prairie voles, in Mongolian gerbils, intra- and intersexual aggression toward unfamiliar conspecifics after bonding has been observed. Female gerbils exhibit aggression toward potential competitors in resident intruder tests and toward unfamiliar males in a partner preference test (Razzoli and Valsecchi, 2006). Following separation from a pair-bond partner for 1 week, male gerbils exhibited a significant decrease in aggressive behaviors to novel males (Hendrie & Starkey, 1998). This may suggest a return to baseline after heightened aggression during pair-bonding maintenance. Consistent with these findings, in the present study we observed a trend ($p = 0.06$) in the social interaction test suggesting that aggressive behavior toward a novel, same-sex conspecific increases the longer a male has been pair-bonded with his mate. However, a similar trend on inter-sexual aggression was absent, suggesting that remaining pair-bonded for over 6 weeks does not necessarily result in increased male aggression toward potential mates in gerbils. Further, we found that when a male gerbil has been separated from his partner for 4 weeks he is more investigative of and more prosocial toward a potential mate than a male that has remained pair-bonded. This suggests that 4 weeks may be sufficient time for a male gerbil to overcome any behavioral and/or neural changes associated with pair-bond maintenance,

thereby allowing a male to interact with a novel female in a manner that could potentially result in the formation of a new pair-bond.

PVN vasopressin and social behavior

Several studies have examined the involvement of PVN VP and social behavior, especially in the context of parental care and bonding. For example, in prairie voles, the duration that mothers spend grooming their pups is inversely associated with the number of VP-ir neurons in the PVN (Hiura et al., 2023). Consistent with this, prairie vole mothers and fathers exhibit less PVN VP neural activity during typical interactions with their pups compared to parents that were separated and then reunited with their pups (Kelly et al., 2017). Together these studies suggest that 'normal' parent-offspring affiliative behaviors may not be regulated by PVN VP. However, studies in mice have shown that VP plays a critical role in social recognition (Bielsky et al., 2004, 2005), an inherent process in the formation of partner preference (Choleris et al., 2009;Young et al., 2005). Although most of the studies exploring effects of VP on social recognition focused on lateral septum, a separate group argued for the importance of PVN to hippocampus CA2 region connectivity through vasopressin 1b receptor (V1b), demonstrating that V1b was critical for social recognition (Smith et al., 2016). Following this line of research, optogenetically stimulating VP releasing synapses (that likely originate in the PVN) in the CA2 of mice during acquisition stage of a memory task drastically improves performance (Smith et al., 2016). Moreover, chemogenetically stimulating the same pathway induces partner preference in C57BL/6J lab mice, a species that normally do not pair bond (Cymerblit-Sabba et al., 2020).

Collectively, these results suggest that the PVN VP neuronal population, and in particular its innervation of the hippocampus, plays a critical role in social recognition and pair-bond formation.

More studies have examined a role for PVN VP in investigative behavior. PVN VP cell ablation in mice increased female social investigation, but did not alter investigation in males (Rigney et al., 2020, 2022; Rigney, Nicole et al., 2019). Because we found here that PVN VP is positively related to investigative behavior in male gerbils, there may be general species differences in PVN VP modulation of investigation and/or context may play a critical role in nonapeptide modulation of investigative behavior. Other studies have found that PVN VP does not relate to investigation; PVN VP cell stimulation in male mice caused self-grooming instead of social interactions with female mice (Islam et al., 2022). Such differences might be an effect of sexually dimorphic connection of PVN VP fibers, which have denser innervation to central areas in females than males (Rigney et al., 2022). However, PVN VP cell density did not differ between sexes in mice (Rood et al., 2013), and no dimorphism is seen in PVN Vasopressin receptor 1a (V1a) receptors in gerbils (Kelly et al., 2021). Interestingly, social recognition and investigation may be regulated by similar mechanisms in the brain, given that delivering selective V1a antagonist to lateral septum impaired social recognition in rats, while delivering V1a receptor agonist decreased investigation behavior (Veenema et al., 2012).

Literature exploring whether PVN VP affects social approach is scarce. Our results indicated that there was no difference in latency to approach between treatment groups and within subjects, suggesting that male gerbil approach behavior is not influenced by pair-bonding status.

However, here we found that PVN VP neuron densities were greater in male gerbils that had been separated from their pair-bond partner. Additionally, PVN VP neuronal densities positively correlated with investigation of a novel, opposite-sex conspecific. This suggests that PVN VP in male gerbils may facilitate investigation, a behavior that is likely necessary for a male to find a new mate after it has recently lost a former partner.

Because PVN VP neurons send axonal projections to multiple parts of the brain to have different effects on behavior, in addition to PVN VP's capability of modulating positive or neutral behavior like social investigation or recognition, there are also studies showing that PVN VP can regulate anti-social behaviors like aggression. For example, dominant, male mandarin voles have greater PVN VP-immunoreactivity (-ir) than subordinates (Qiao et al., 2014), and aggressive behavior is positively correlated with greater PVN VP expression in Brandt's voles (Huang et al., 2021). In male song sparrows, PVN VP cells are more active after agnostic encounters (Goodson and Evans, 2004). Further, in lizards, PVN VP cell activity is positively correlated with aggression and can predict subordinate status (Kabelik et al., 2013). Lastly, VP knockdown in the PVN of zebra finches produced a sexually dimorphic effect on aggression toward opposite-sex conspecifics, yet did not influence aggression toward same-sex conspecifics (Kelly and Goodson, 2014).

Despite correlational studies suggesting involvement of PVN VP in aggression in rodents, ablating PVN VP cells in mice did not alter resident-intruder aggression (Rigney et al., 2019). Together these studies suggest that the PVN VP neuronal population is involved in modulating aggression in at least some contexts. In the present study, we did not observe high levels of

aggression from male gerbils. Indeed, in the final social interaction IEG study in which male subjects interacted with a novel female, less than half of the males exhibited any aggression at all, potentially explaining why we did not observe a relationship between PVN VP and aggressive behavior.

PVN vasopressin and stress

Activation of the hypothalamic-pituitary-axis (HPA) is important for regulating the stress response in face of homeostatic challenges (Herman et al., 2016). While most of the PVN VP neurons are present in the magnocellular subdivision of the PVN projecting to the posterior pituitary, there are parvocellular neurons that produce corticotropin-releasing hormone (CRH) that projects to median eminence, where both CRH and VP are co-released into the pituitary portal circulation. Importantly, a subset of CRH producing PVN neurons also coexpress VP (Itoi et al., 2014). In colchicine-pretreated rats, colocalization is more profound at median eminence, and repeated exposure to immobilization stress can make nearly all CRH positive neurons to be also VP positive (Bartanusz et al., 1993), demonstrating that PVN VP can also play a critical role in modulating a stress response. Consistent with this, an experiment in Wistar rats reported VP release in the PVN after social defeat (Ebner et al., 2005; Wotjak et al., 1996). Inhibiting PVN VP and CRH receptors through inverse microdialysis caused significant increase in the plasma adrenocorticotropic hormone (ACTH) concentration, suggesting an inhibition effect alleviated upon imminent stress, in this case social defeat (Wotjak et al., 1996). Cre- driven deletion of PVN VP neurons in mice resulted in a sexually dimorphic effect, in which males exhibited an increase in non-social anxiety-related behaviors in the elevated-plus maze (Rigney et al., 2021),

again suggesting that this neuronal population may play a particularly important role in stress-related behaviors in males. Taken together, the PVN exhibits robust anatomical connections and is involved in responding to stress in addition to modulating social behavior.

A previous study in prairie voles found that partner loss increased the density of oxytocin-immunoreactivity (-ir), VP-ir, and CRH-ir cells in the PVN (Sun et al., 2014). This finding mirrors what we found here in male gerbils, suggesting that the loss of a pair-bond partner may have similar effects on PVN VP across several socially monogamous species. Because PVN VP increases in response to stressors in many species, it is possible that an increase in PVN VP expression after partner loss may reflect the stress of losing a partner and having a bond erode. Alternatively, our finding that separated males have more PVN VP neurons might reflect an effect of stress response due to 4 weeks of social isolation rather than specifically due to the loss of a partner or bond erosion. Consistent with this, a study in prairie voles found that in juvenile subjects (postnatal day 9) that were isolated, but not those who stayed together with their parents and siblings or reunited after isolation, PVN VP-ir neurons were significantly higher (Kelly et al., 2018). This suggests a rapid synthesis of VP in response to social isolation. Because PVN VP is known for modulating a stress response, and because social isolation is likely stressful to a socially monogamous gerbil, the increase in PVN VP neurons observed here could at least partially reflect the stress of isolation.

CONCLUSION

Here we examined how partner separation influenced the brain and behavior of male Mongolian gerbils. Partner separation did not globally influence all behavior: anxiety related behavior in a non-social context, the latency to approach an opposite sex conspecifics, and behavior toward same-sex conspecifics did not differ based on pair-bond status. However, males that were separated from their partner exhibited more prosocial and more investigative behavior toward a novel female, suggesting that after 4 weeks of losing a partner, a male gerbil may be more amenable toward exploring a potential new mating opportunity. Further, males that were separated from their partner had more PVN VP neurons than pair-bonded males. Because males that have more PVN VP spend more time investigating a novel female, PVN VP may facilitate investigative behavior. Therefore, when a male loses their pairbond partner and becomes separated/single, there's an increase in PVN VP cell density, possibly to promote investigative behavior in a reproductive context by the male so that he will be more likely to find a new mate. An increase in PVN VP densities may also reflect the stress of social isolation and/or bond loss, however PVN VP-mediated investigative behavior could serve to alleviate such stress by driving an animal toward a social encounter to avoid isolation.

Reference

Bales KL, Ardekani CS, Baxter A, Karaskiewicz CL, Kuske JX, Lau AR, Savidge LE, Sayler KR, Witczak LR. What is a pair bond? *Horm Behav.* 2021 Nov;136:105062. doi: 10.1016/j.yhbeh.2021.105062. Epub 2021 Sep 30. PMID: 34601430.

Bartanusz V, Jezova D, Bertini LT, Tilders FJH, Aubry J-M, Kiss JZ. Stress-induced increase in vasopressin and corticotropin-releasing factor expression in hypophysiotropic paraventricular neurons. *Endocrinology* 1993; **132:** 895-902.

Bielsky, I. F., Hu, S. B., Ren, X., Terwilliger, E. F., & Young, L. J. (2005). The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study. *Neuron*, *47*(4), 503–513. https://doi.org/10.1016/j.neuron.2005.06.031

Bielsky IF, Hu SB, Szegda KL, Westphal H, Young LJ. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacology*. 2004 Mar;29(3):483-93. doi: 10.1038/sj.npp.1300360. PMID: 14647484.

Blocker TD, Ophir AG, 2016. A preference to bond? Male prairie voles form pair bonds even in the presence of multiple receptive females. *Anim Behav* 122:89–97.

Blumenthal SA, Young LJ. The Neurobiology of Love and Pair Bonding from Human and *Animal Perspectives*. Biology. 2023; 12(6):844.https://doi.org/10.3390/biology12060844

Cho, M. M., DeVries, A. C., Williams, J. R., & Carter, C. S. (1999). The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus* ochrogaster). *Behavioral Neuroscience*, *113*(5), 1071–1079. https://doi.org/10.1037/0735-7044.113.5.1071

Cui, Z., Gerfen, C. R., & Young 3rd, W. S. (2013). Hypothalamic and other connections with dorsal CA2 area of the mouse hippocampus. *Journal of Comparative Neurology*, *521*(8), 1844–1866. https://doi.org/10.1002/cne.23263

Cymerblit-Sabba, A., Smith, A. S., Williams Avram, S. K., Stackmann, M., Korgan, A. C., Tickerhoof, M. C., & Young, W. S. (2020). Inducing Partner Preference in Mice by Chemogenetic Stimulation of CA2 Hippocampal Subfield. *Frontiers in Molecular Neuroscience*, *13*. https://doi.org/10.3389/fnmol.2020.00061

Ebner K, Wotjak CT, Landgraf R, Engelmann M. Neuroendocrine and behavioral response to social confrontation: residents versus intruders, active versus passive coping styles. *Horm. Behav* (2005) 47:14–21.

Freeman, S. M., & Young, L. J. (2016). Comparative perspectives on oxytocin and vasopressin receptor research in rodents and primates: Translational implications. *Journal of Neuroendocrinology*, *28*(4). https://doi.org/10.1111/jne.12382

Fricker, B.A., Roshko, V.C., Jiang, J. *et al.* Partner separation rescues pair bond-induced decreases in hypothalamic oxytocin neural densities. *Sci Rep* **13**, 4835 (2023). https://doi.org/10.1038/s41598-023-32076-8

Getz, L. L., & Carter, C. S. (1980). Social organization in *Microtus* ochrogaster populations. *Biologist*, *62*(1-4), 56-69.

Getz, L. L., Carter, C. S., & Gavish, L. (1981). The mating system of the prairie vole, *Microtus* ochrogaster: field and laboratory evidence for pair-bonding. *Behavioral Ecology and Sociobiology*, 189-194.

Gobrogge, K. L., Liu, Y., Jia, X., & Wang, Z. (2007). Anterior hypothalamic neural activation and neurochemical associations with aggression in pair-bonded male prairie voles. *The Journal of comparative neurology*, 502(6), 1109–1122. https://doi.org/10.1002/cne.21364

Goodson, J. L., & Evans, A. K. (2004). Neural responses to territorial challenge and nonsocial stress in male song sparrows: segregation, integration, and modulation by a vasopressin V1 antagonist. *Hormones and behavior*, *46*(4), 371–381. https://doi.org/10.1016/j.yhbeh.2004.02.008

Hendrie, C. A., & Starkey, N. J. (1998). Pair-bond disruption in Mongolian gerbils: Effects on subsequent social behaviour. *Physiology & Behavior*, *63*(5), 895–901. https://doi.org/10.1016/s0031-9384(98)00005-5

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Comprehensive Physiology*, 6(2), 603–621. https://doi.org/10.1002/cphy.c150015

Hiura, L. C., Lazaro, V. A., & Ophir, A. G. (2023). Plasticity in parental behavior and vasopressin: responses to co-parenting, pup age, and an acute stressor are experience-dependent. *Frontiers in behavioral neuroscience*, *17*, 1172845. https://doi.org/10.3389/fnbeh.2023.1172845

Huang, S., Li, G., Pan, Y., Liu, J., Zhao, J., Zhang, X., Lu, W., Wan, X., Krebs, C. J., Wang, Z., Han, W., & Zhang, Z. (2021). Population variation alters aggression-associated oxytocin and vasopressin expressions in brains of Brandt's voles in field conditions. *Frontiers in zoology*, *18*(1), 56. https://doi.org/10.1186/s12983-021-00441-w

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: Behavioral consequences. *Physiology & Behavior*, *57*(4), 615–627. https://doi.org/10.1016/0031-9384(94)00362-9

Islam, M. T., Maejima, T., Matsui, A., & Mieda, M. (2022). Paraventricular hypothalamic vasopressin neurons induce self-grooming in mice. *Molecular Brain*, *15*(1), 47. https://doi.org/10.1186/s13041-022-00932-9

Kabelik, D., Alix, V. C., Burford, E. R., & Singh, L. J. (2013). Aggression- and sex-induced neural activity across vasotocin populations in the brown anole. *Hormones and behavior*, *63*(3), 437–446. https://doi.org/10.1016/j.yhbeh.2012.11.016

Kelly, A., Gonzalez Abreu, J., & Thompson, R. (2022). Beyond sex and aggression: Testosterone rapidly matches behavioural responses to social context and tries to predict the future. *Proceedings of the Royal Society B: Biological Sciences*, 289(1976). https://doi.org/10.1098/rspb.2022.0453

Kelly, A. M., Hiura, L. C., & Ophir, A. G. (2018). Rapid nonapeptide synthesis during a critical period of development in the prairie vole: plasticity of the paraventricular nucleus of the hypothalamus. *Brain structure & function*, *223*(6), 2547–2560. https://doi.org/10.1007/s00429-018-1640-2

Kelly, A. M., Hiura, L. C., Saunders, A. G., & Ophir, A. G. (2017). Oxytocin Neurons Exhibit Extensive Functional Plasticity Due To Offspring Age in Mothers and Fathers. *Integrative and comparative biology*, *57*(3), 603–618. https://doi.org/10.1093/icb/icx036

Kelly A.M. and Seifert A.W. (2021) Distribution of vasopressin and oxytocin neurons in the basal forebrain and midbrain of spiny mice (*Acomys* cahirinus), *Neuroscience*, 468, 16-28.

Kenkel, W. M., Perkeybile, A. M., Yee, J. R., & Carter, C. S. (2019). Rewritable fidelity: How repeated pairings and age influence subsequent pair-bond formation in male prairie voles. *Hormones and Behavior*, *113*, 47–54. https://doi.org/10.1016/j.yhbeh.2019.04.015

Lim MM, Wang Z, Olazábal DE, Ren X, 2004. Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature* 429:754.

Mabry KE, Streatfeild CA, Keane B, Solomon NG, 2011. *avpr1a* length polymorphism is not associated with either social or genetic monogamy in free-living prairie voles. *Anim Behav* 81:11–18.

Maestripieri, D. (1992). Functional aspects of maternal aggression in mammals. *Canadian journal of zoology*, *70*(6), 1069-1077.

Ophir AG, Campbell P, Hanna K, Phelps SM, 2008. Field tests of *cis*-regulatory variation at the prairie vole *avpr1a* locus: association with V1aR abundance but not sexual or social fidelity. *Horm Behav* 54:694–702.

Pedersen, C. A., Ascher, J. A., Monroe, Y. L., & Prange, A. J., Jr (1982). Oxytocin induces maternal behavior in virgin female rats. *Science (New York, N.Y.)*, *216*(4546), 648–650. https://doi.org/10.1126/science.7071605

Piña-Andrade, S., Ramos, G., Cárdenas-León, M., Martínez, A., Romero-Morales, L., Martínez-Torres, M., Cedillo-Ildefonso, B., & Luis, J. (2020). Testosterone dependent

territorial aggression is modulated by cohabitation with a female in male Mongolian gerbils (meriones unguiculatus). *Hormones and Behavior*, *117*, 104611. https://doi.org/10.1016/j.yhbeh.2019.104611

Qiao, X., Yan, Y., Wu, R., Tai, F., Hao, P., Cao, Y., & Wang, J. (2014). Sociality and oxytocin and vasopressin in the brain of male and female dominant and subordinate mandarin voles. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology*, *200*(2), 149–159. https://doi.org/10.1007/s00359-013-0870-2

Rigney, N., de Vries, G. J., Petrulis, A., & Young, L. J. (2022). Oxytocin, Vasopressin, and Social Behavior: From Neural Circuits to Clinical Opportunities. *Endocrinology*, *163*(9), bqac111. https://doi.org/10.1210/endocr/bqac111

Rigney, N., Whylings, J., de Vries, G. J., & Petrulis, A. (2020). Sex Differences in the Control of Social Investigation and Anxiety by Vasopressin Cells of the Paraventricular Nucleus of the Hypothalamus. *Neuroendocrinology*, *111*(6), 521–535. https://doi.org/10.1159/000509421

Rigney, N., Whylings, J. Mieda, M., de Vries, G. J., & Petrulis, A. (2019). Sexually Dimorphic Vasopressin Cells Modulate Social Investigation and Communication in Sex-Specific Ways. *Eneuro*, *6*(1), ENEURO.0415-18.2019. https://doi.org/10.1523/ENEURO.0415-18.2019

Rigney, N., Zbib, A., de Vries, G. J., & Petrulis, A. (2022). Knockdown of sexually differentiated vasopressin expression in the bed nucleus of the stria terminalis reduces social and sexual behaviour in male, but not female, mice. *Journal of Neuroendocrinology*, *34*(9), e13083. https://doi.org/10.1111/jne.13083

Rigney N, Whylings J, de Vries GJ, Petrulis A. Sex differences in the control of social investigation and anxiety by vasopressin cells of the paraventricular nucleus of the hypothalamus. *Neuroendocrinology*. (2021) 111(6):521–35. doi: 10.1159/000509421.

Rood BD, Stott RT, You S, Smith CJW, Woodbury ME, De Vries GJ. Site of origin of and sex differences in the vasopressin innervation of the mouse (Mus musculus) brain. *J Comp Neurol*. 2013;521(10):2321-2358. doi: 10.1002/cne.23288

Sadino, J. M., & Donaldson, Z. R. (2018). Prairie voles as a model for understanding the genetic and epigenetic regulation of attachment behaviors. *ACS Chemical Neuroscience*, *9*(8), 1939–1950. https://doi.org/10.1021/acschemneuro.7b00475

Sadino, J. M., & Donaldson, Z. R. (2018). Prairie Voles as a Model for Understanding the Genetic and Epigenetic Regulation of Attachment Behaviors. *ACS chemical neuroscience*, 9(8), 1939–1950. https://doi.org/10.1021/acschemneuro.7b00475

Smith, A., Williams Avram, S., Cymerblit-Sabba, A. *et al.* Targeted activation of the hippocampal CA2 area strongly enhances social memory. *Mol Psychiatry* 21, 1137–1144 (2016). https://doi.org/10.1038/mp.2015.189

Smith, A. S., Williams Avram, S. K., Cymerblit-Sabba, A., Song, J., & Young, W. S. (2016). Targeted activation of the hippocampal CA2 area strongly enhances social memory. *Molecular Psychiatry*, *21*(8), 1137–1144. https://doi.org/10.1038/mp.2015.189

Solomon NG, Richmond AR, Harding PA, Fries A, Jacquemin S et al. ., 2009. Polymorphism at the *avpr1a* locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Mol Ecol* 18:4680–4695.

Sun, P., Smith, A. S., Lei, K., Liu, Y., & Wang, Z. (2014). Breaking bonds in male prairie vole: long-term effects on emotional and social behavior, physiology, and neurochemistry. *Behavioural Brain Research*, 265, 22–31. https://doi.org/10.1016/j.bbr.2014.02.016

Tchabovsky, A. V., Savinetskaya, L. E., Ovchinnikova, N. L., Safonova, A., Ilchenko, O. N., Sapozhnikova, S. R., & Vasilieva, N. A. (2019). Sociability and pair-bonding in gerbils: A comparative experimental study. *Current Zoology*, 65(4), 363–373. https://doi.org/10.1093/cz/zoy078

Tchabovsky, A. V., Savinetskaya, L. E., Ovchinnikova, N. L., Safonova, A., Ilchenko, O. N., Sapozhnikova, S. R., & Vasilieva, N. A. (2018). Sociability and pair-bonding in gerbils: A comparative experimental study. *Current Zoology*, *65*(4), 363–373. https://doi.org/10.1093/cz/zoy078

Thomas, J. A., & Birney, E. C. (1979). Parental care and mating system of the prairie vole, *Microtus* ochrogaster. *Behavioral Ecology and Sociobiology*, 171-186.

Trivers, R. (1972). *Parental investment and sexual selection*. Biological Laboratories, Harvard University.

Veenema, A. H., Bredewold, R., & De Vries, G. J. (2012). Vasopressin regulates social recognition in juvenile and adult rats of both sexes, but in sex- and age-specific ways. *Hormones and behavior*, *61*(1), 50–56. https://doi.org/10.1016/j.yhbeh.2011.10.002

Walum, H., & Young, L. J. (2018). The neural mechanisms and circuitry of the pair bond. *Nature Reviews Neuroscience*, *19*(11), 643–654. https://doi.org/10.1038/s41583-018-0072-6

Wersinger, S. R., Kelliher, K. R., Zufall, F., Lolait, S. J., O'Carroll, A. M., and Young, W. S. III. (2004). Social motivation is reduced in vasopressin 1b receptor null mice despite normal performance in an olfactory discrimination task. *Horm. Behav.* 46, 638–645. doi: 10.1016/j.yhbeh.2004.07.004

Winslow JT, Hastings N, Carter CS, Harbaugh CR, & Insel TR (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature*, 365, 545–548.

Witt, D. M., Carter, C. S., Chayer, R., & Adams, K. (1990). Patterns of behaviour during postpartum oestrus in prairie voles, *Microtus* ochrogaster. *Animal Behaviour*, *39*(3), 528-534.

Wotjak, C. T., Kubota, M., Liebsch, G., Montkowski, A., Holsboer, F., Neumann, I., & Landgraf, R. (1996). Release of vasopressin within the rat paraventricular nucleus in response to emotional stress: a novel mechanism of regulating adrenocorticotropic hormone secretion?. *The Journal of Neuroscience*, 16(23), 7725–7732. https://doi.org/10.1523/JNEUROSCI.16-23-07725.1996

Young, K. A., Liu, Y., & Wang, Z. (2008). The Neurobiology of Social Attachment: A Comparative Approach to behavioral, neuroanatomical, and neurochemical studies. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *148*(4), 401–410. https://doi.org/10.1016/j.cbpc.2008.02.004

Young, L. J., Murphy Young, A. Z., & Hammock, E. A. D. (2005). Anatomy and neurochemistry of the pair bond. *Journal of Comparative Neurology*, *493*(1), 51–57. https://doi.org/10.1002/cne.20771

Yu, P., Zhang, M., Nan, X., Zhao, H., & Gong, D. (2020). Differences in the number of oxytocin, vasopressin, and tyrosine hydroxylase cells in brain regions associated with mating among great, midday, and Mongolian gerbils. *Brain Research*, *1733*, 146677. https://doi.org/10.1016/j.brainres.2020.146677

Zhang, L., & Hernández, V. S. (2013). Synaptic innervation to rat hippocampus by vasopressin-immuno-positive fibres from the hypothalamic supraoptic and paraventricular nuclei. *Neuroscience*, *228*, 139–162. https://doi.org/10.1016/j.neuroscience.2012.10.010