NEUROSCIENCE
RESEARCH ARTICLE
N. S. Lee et al. / Neuroscience 515 (2023) 62–70

# Peer Social Environment Impacts Behavior and Dopamine D1 Receptor Density in Prairie Voles (*Microtus ochrogaster*)

**Nicole S. Lee,** [a,c] **Claire Y. Kim** [b] **and Annaliese K. Beery** [a,b,c*]

[a] *Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003, USA*

[b] *Neuroscience Program, Department of Psychology, Smith College, Northampton, MA 01063, USA*

[c] *Department of Integrative Biology, UC Berkeley, Berkeley, CA 94720, USA*

**Abstract—Prairie voles (*Microtus ochrogaster*) are socially monogamous rodents that form selective, long-lasting relationships with mates and with same-sex peers. It is unknown to what extent mechanisms supporting 'peer relationships' are similar to those involved in mate relationships. The formation of pair bonds is dependent on dopamine neurotransmission, whereas the formation of peer relationships is not, providing evidence of relationship type-specificity. The current study assessed endogenous structural changes in dopamine D1 receptor density in male and female voles across different social environments, including long-term same-sex partnerships, new same-sex partnerships, social isolation, and group housing. We also related dopamine D1 receptor density and social environment to behavior in social interaction and partner preference tests. Unlike prior findings in mate pairs, voles paired with new same-sex partners did not exhibit upregulated D1 binding in the nucleus accumbens (NAcc) relative to controls paired from weaning. This is consistent with differences in relationship type: D1 upregulation in pair bonds aids in maintaining exclusive relationships through selective aggression, and we found that formation of new peer relationships did not enhance aggression. Isolation led to increases in NAcc D1 binding, and even across socially housed voles, individuals with higher D1 binding exhibited increased social avoidance. These findings suggest that elevated D1 binding may be both a cause and a consequence of reduced prosociality. These results highlight the neural and behavioral consequences of different non-reproductive social environments and contribute to growing evidence that the mechanisms underlying reproductive and non-reproductive relationship formation are distinct. Elucidation of the latter is necessary to understand mechanisms underlying social behavior beyond a mating context.**

**Key words: prairie vole, dopamine, peer affiliation, social isolation, group housing, social environment.**

## INTRODUCTION

Relationships between same-sex conspecifics play an essential role in the lives of group-living animals. Yet, the pathways supporting non-reproductive peer relationships are not well understood. Prairie voles (*Microtus ochrogaster*) are socially monogamous rodents that form selective social relationships with opposite-sex mates (reviewed in Gobrogge and Wang, 2016; Carter, 2017; Walum and Young, 2018) as well as same-sex peers (DeVries et al., 1997; Beery et al., 2018; Lee et al., 2019). They provide an ideal opportunity to compare mechanisms underlying selective social relationships in both reproductive (mate) and non-reproductive (peer) contexts. Here, we investigate the role of social environment (including pair housing, extended isolation, new same-sex pairings, and housing in same-sex groups) on social behavior and endogenous regulation of dopamine receptor binding.

Mate relationships in prairie voles are highly rewarding (Goodwin et al., 2019; Beery et al., 2021; Vahaba et al., 2022), and this is true to some extent for peer relationships as well. Non-reproductive relationships with familiar same-sex peers are strongly rewarding in female prairie voles, who will expend substantial effort to reach and huddle with a peer companion, while males do not appear to find same-sex relationships reinforcing (Beery et al., 2021). Social motivation for mates also appears to be more durable than for peers, as prairie voles display socially conditioned place preferences for environments associated with mates or new peer companions, but not long-term peer partners (Goodwin et al., 2019; Lee et al., 2019). Nonetheless, both sexes appear to reap benefits from these relationships. Separation from

*Correspondence to: A.K. Beery, Department of Integrative Biology, 3040 VLSB #3140, Berkeley, CA 94720, USA.
E-mail address: abeery@berkeley.edu (A. K. Beery).

*Abbreviations:* NAcc, nucleus accumbens; CPu, caudoputamen; PPT, partner preference test; SI, social interaction test; PC, principal component.

https://doi.org/10.1016/j.neuroscience.2023.02.002
0306-4522/

same-sex peers triggers anxiety- and depression-like behaviors in female and male prairie voles (Grippo et al., 2007, 2008; Lieberwirth et al., 2012), and social buffering attenuates anxiety- and depression-like behaviors in stressed animals (Burkett et al., 2016). In these studies, same-sex conspecifics provided social buffering, and removal of same-sex cage-mates induced the negative effects of social isolation.

The dopamine system is highly involved in reward, motivation, learning, and motor activity, with D1-like and D2-like receptors often contributing differently to specific functions (e.g., Surmeier et al., 2007). For example, it has been suggested that D1 receptors are involved in learning reward-related behaviors while D2 receptors are involved in aversion-related behaviors (Kravitz et al., 2012; Yawata et al., 2012;Verharen et al., 2019). Whereas D1 receptors alone are essential for maternal motivation in rats (Numan et al., 2005; Stolzenberg et al., 2007), both D1 and D2 receptors are involved in maternal memory (Parada et al., 2008). And, whereas D1 receptors regulate food anticipatory activity in mice (Gallardo et al., 2014), overexpression of D2 receptors instead decreases food anticipatory activity by decreasing motivation (LeSauter et al., 2020).

Dopamine signaling plays a prominent role in the formation and maintenance of prairie vole pair bonds, with distinct roles of D1 and D2 receptors. The dopamine receptor antagonist haloperidol blocks partner preference formation in prairie vole mates (Wang et al., 1999), and activation of both dopamine and oxytocin receptors is necessary for pair bond formation (Liu and Wang, 2003). Dopamine signaling at D2 receptors in the nucleus accumbens is particularly important for pair bond formation in male and female prairie voles (Wang et al., 1999; Gingrich et al., 2000; Aragona et al., 2003; Liu and Wang, 2003), while pair bond maintenance in male prairie voles is mediated by upregulation of dopamine D1 receptors in the nucleus accumbens (Aragona et al., 2006). D1 receptor signaling may promote pair bonding in part through its role in promoting selective aggression toward non-partners following pair bonding (Aragona and Wang, 2009). Maintenance of pair bonds is also associated with upregulated mRNA expression of genes encoding dopamine D1 receptors in males and females (Resendez et al., 2016).

In contrast to mate relationships, dopamine signaling is not necessary for the formation of peer relationships in prairie voles, although it can facilitate social reward (Lee and Beery, 2021). The roles of dopamine and social reward in partner preference thus appear to differ by relationship type. However, pharmacological manipulation of dopamine signaling does not provide insight into the endogenous, long-term, structural changes that occur during the onset and maintenance of peer relationships.

We test the hypothesis that dopaminergic regulation of social bond maintenance, rather than social bond formation, may underlie shared characteristics with prairie vole pair bonds—that is, high selectivity for familiar partners over strangers. Specifically, we assessed the effects of different social environments on dopamine D1 receptor density and social behavior toward both familiar peers and unfamiliar 'strangers'. We quantified social behavior and receptor binding in male and female prairie voles housed alone for an extended interval, in long-term established same-sex pairs, and in females re-paired with new same-sex partners in adulthood or housed in groups of five females. This study contributes to our understanding of how reproductive pair bonds may differ from non-reproductive peer relationships, and of the neural and behavioral consequences of different social environments in a selectively social species.

## EXPERIMENTAL PROCEDURES

### Animal subjects

Prairie voles from our in-house breeding colony were group weaned at 21 ± 1 days, then placed in pairs or groups with same-sex sibling(s) or age-matched non-sibling(s) within one week. Voles were maintained on a long day (LD) light cycle (14 h light; 03:00 to 17:00 EST). Subjects were housed in clear plastic cages (45 × 25 × 15 cm for 1–2 voles; 51 × 41 × 20.5 cm for groups of five voles) with aspen bedding (Harlan TekLab), nesting material (Lab Supply Enviro-dri and a nestlet), and an opaque plastic hiding tube. Food (Labdiet Mouse Chow 5015 supplemented with Labdiet Rabbit Chow 5326) and water were available *ad libitum*, with every-other-day supplementation with fresh produce (apple or carrot). Room temperature was maintained at ~20 °C. All procedures adhered to federal and institutional guidelines and were approved by the Institutional Animal Care and Use Committee.

### Experimental design

Male and female prairie voles ($n$ = 12–15/group) were assigned to one of four groups. ''Control" animals were maintained in same-sex pairs from weaning (Fig. 1), and groups were formed from mixed-litter female quintets (''group living"). ''Isolated" animals were separated to solo-housing for 4 weeks prior to the start of behavioral testing at d80 ± 7, while voles in the ''re-paired" group were separated from their first cage-mates for one week, then placed with new same-sex partners for two weeks prior to testing. Males were not included in the group-living and re-paired conditions because male prairie voles exhibit more aggressive behaviors toward same-sex conspecifics than do females, especially upon re-pairing in adulthood (Lee et al., 2019).

All individuals in the control, re-paired, and isolation groups underwent social interaction (SI) tests at d80 ± 7. Two to four days after social interaction testing, partner preference tests (PPT) were conducted. In the control and re-paired groups, both voles in each pair served as focal animals and as partners in consecutive PPTs spaced 2–4 days apart. In the isolation group, voles served as strangers once in PPTs (during either PPT session 1 or 2 for the other groups), in order to control for tethering exposure in other groups. Four voles from each quintet served as focal animals in social interaction and partner preference

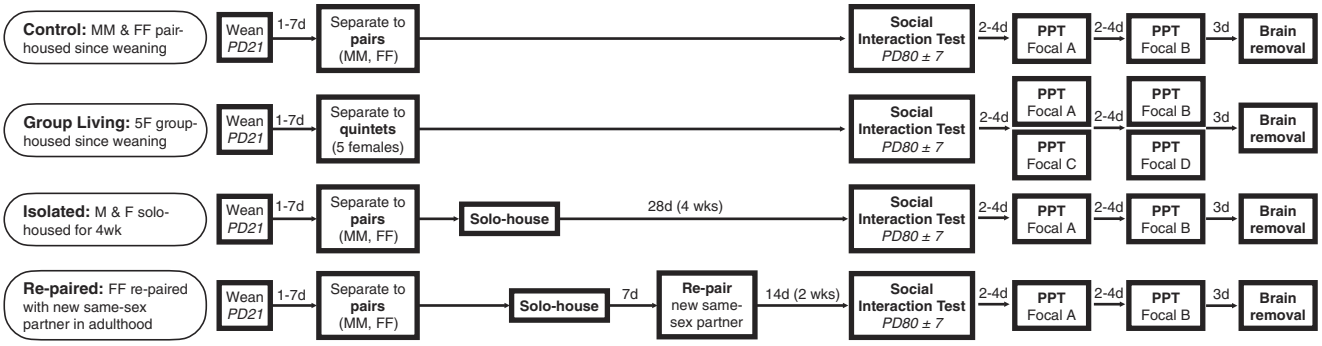


**Fig. 1.** Experimental design. Groups consisted of voles in long-term same-sex pair housing (controls), isolated males and females, females re-paired with a new peer partner in adulthood, and females housed in social groups (of 5). Only females were included in the latter two groups because of higher rates of aggression in adult males. In the isolation group, both voles in each pair served as focal animals in social interaction tests and served as strangers once in other groups' partner preference tests. In group-living voles, four of the five voles served as focal animals in SI tests and PPTs, with round robin testing ensuring that each of the four voles served as a focal once and as a stranger once.

tests. Round robin testing was used for PPTs such that each of the four voles served as a focal once and as a stranger once. For example, in PPT session 1, focal A was tested with B as a partner, then focal B was tested with C as a partner in the second PPT session. In parallel, focal C was tested with D as a partner in PPT session 1, then focal D was tested with A as a partner in PPT session 2. Three days after PPT session 2, voles were euthanized and their brains were removed for receptor autoradiography.

### Social interaction test

Interactions with an unfamiliar vole were assessed in a neutral arena, as aggression between conspecifics in prairie voles is as high in a neutral arena as it is in home-cage resident intruder tests (Harper and Batzli, 1997). The focal vole was placed in a new cage and allowed to acclimate for 10 min. An unrelated, unfamiliar same-sex stranger was marked with orange chalk (ground with water into a thick paste) for identification, then introduced into the cage. The test was recorded for 10 min, or was terminated early if the experimenters determined that voles were at risk of injury (17 terminated early/80 total tests). Tests were scored by an observer unaware of subject group, using BORIS (Friard and Gamba, 2016) to measure the frequency and duration of behaviors. Test scoring focused on the behavior of the focal vole and included measurements of aggressive and social behaviors. Aggressive behaviors quantified included latency to first physical attack, lateral attack/threat, upright (boxing), chasing, lunge, and clinch (as in Koolhaas et al., 2013; Lee et al., 2019). Clinch refers to a behavior in which the voles scuffle but are not upright, with one vole supine and the other on top. Lunge refers to a behavior where one vole moves quickly to attack another but fails to make contact, unlike lateral attack, upright, and clinch. Social and investigative behaviors included sniffing, grooming, and huddling. Autogrooming and flight were also recorded. In tests where chalk marks were not consistently visible throughout the recording, only reciprocal behaviors (i.e., huddling time) and latency to first physical attack were measured. Because some tests were terminated early, a maximum test duration that included nearly all animals (3:41, excludes three tests) was used to cap analysis of all measures except for latency to first physical attack.

### Peer partner preference test

Peer partner preference testing was conducted as a classic partner preference test (Williams et al., 1992), but with same-sex partners and strangers (DeVries et al., 1997; Beery et al., 2018; Lee et al., 2019). Testing occurred in a linear apparatus (75 × 20 × 30 cm) divided into three equal-sized chambers, according to the protocol detailed in Beery (2021a). The cage-mate of the focal vole (the partner) was tethered at one end of the apparatus, and an age-matched, unrelated, same-sex novel vole (the stranger) was tethered at the other end. Strangers were pair-housed from weaning and were tethered no more than three times each. The focal vole was placed in the center chamber and allowed to move freely for the duration of the 180-minute test. Tests were video recorded, and trained observers ($r \geq 0.97$ between any two scorers on training videos) used a custom scoring script (IntervoleTimer1.6.pl, Beery, 2021b) to quantify the amount of time focal voles spent huddling (side-by-side or one on top of the other), duration in each chamber, and number of times the focal vole crossed between chambers. Scorers were unaware of subject treatment and position of the partner/stranger.

### Receptor autoradiography

Following sacrifice, brains were removed, rapidly frozen on crushed dry ice, and stored at −80 °C until cryosectioning. Brains were sectioned coronally at 20 μm on a cryostat and thaw-mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA, USA) in five parallel series at 100 μm intervals. Frozen sections were placed in racks and thawed until dry, then fixed for 2–7 min in fresh, chilled 0.1% paraformaldehyde (0.1% paraformaldehyde in 0.1 M PBS). Sections were rinsed for 2 × 10 min in 50 mM Tris (pH 7.4), then incubated for 90 min at room temperature in a solution containing the radioligand for that assay (50 mM Tris, 10 mM MgCl2, 0.1% BSA, radioligand). All slides were rinsed for 3 × 5 min in chilled Tris-MgCl2 (50 mM Tris, 10 mM

MgCl2, pH 7.4), dipped in cold distilled water, and air dried.

D1 receptor density was assessed using the tritiated ligand $^{3H}$SCH23390 (PerkinElmer #NET930250UC, Waltham, MA, USA) at a concentration of 4.4 nM, and 1 μm ketanserin was used to prevent additional binding at 5-$HT_2$ receptors (Mansour et al., 1990; Homberg et al., 2016; Mosher et al., 2018). Nonspecific binding was assessed by incubating adjacent sections in 4.4 μM of the selective D1 receptor agonist SKF38393 along with $^{3H}$SCH23390 and ketanserin (Dalton and Zavitsanou, 2011; Mosher et al., 2018), which was effective at eliminating binding at the D1 receptor (Fig. 3(**B,C**)). Concentrations of tritiated ligands were chosen to be at approximately three times their $K_d$ values (as in Mansour et al., 1990).

Slides were apposed to BioMax MR film (Carestream Health) for visualization for 23 days, alongside $^3$H-labeled radiographic standards (Range: 0–489.1 nCi/mg, American Radiolabeled Chemicals, #ART0123, St. Louis, MO, USA). Receptor binding was quantified in the nucleus accumbens (NAcc) core and shell as the primary focus of investigation, and in the adjacent caudoputamen (CPu) as a control, using MCID Analysis 7.0 (InterFocus Imaging ltd., Cambridge, England). Specific binding in each region was quantified bilaterally in three adjacent sections by subtracting non-specific binding from total binding; values were averaged for each subject in each region.

### Statistical analyses

Because males were present in some but not all groups, we analyzed the main effects of group (control, isolated, re-paired and group living) in females, and conducted a separate analysis of sex*group in groups with both sexes (control and isolated). Tests were conducted as ANOVA on single outcomes, or MANOVA on D1 binding in the NAcc and CPu. Significant model results were followed by post-hoc comparisons between groups, as described in the text. Pearson's correlation coefficient was calculated as a measure of the linear association between variables.

Principal components analysis (PCA) was used to reduce behavioral outcomes in the PPT and SI tests to principal components (PCs). Specific PCs are described in the results. Principal components with eigenvalues above 1 (Kaiser criterion; two in each test) were analyzed with regard to other study variables. Peer partner preference within groups was defined as significantly more time huddling with the partner than with the stranger.

Statistical analyses were performed in JMP 14 (SAS) and Prism 8 (GraphPad Software), all tests were two-tailed, and results were deemed significant at $p < 0.05$.

## RESULTS

### Principal components of social behaviors

PCA of behaviors in the partner preference test and social interaction test reduced numerous, sometimes-correlated measures to two PCs. For the PPT, behaviors analyzed included partner huddling, stranger huddling, time in the partner chamber, time in the stranger chamber, and time alone in the neutral chamber. Two PCs explained 95% of the variance. PC1 (71%, eigenvalue 3.56) was characterized by partner selectivity, with factor loadings strongly influenced by partner and stranger variables in opposing directions. PC2 (24%, eigenvalue 1.22) was characterized by sociability, and was most strongly influenced by the inverse of time spent alone. For the SI test, prosocial time (huddling and allogrooming), autogrooming, latency to first physical attack, and frequency of aggressive behaviors (chase, upright, lateral attack, clinch, and lunge) were analyzed. Three PCs explained 87% of the variance. PC1 (37%, eigenvalue 1.48) was characterized by aggression, with latency to aggression and frequency of aggression ranking most strongly. PC2 (33%, eigenvalue 1.32) was most strongly influenced by grooming oriented toward oneself (autogrooming) versus the stranger (allogrooming, negatively loaded). PC3 (17%, eigenvalue 0.68) was influenced by autogrooming and allogrooming in the same direction, but did not meet the criterion for inclusion.

### Effects of social housing on behavior

Social environment (pair-housed, group-housed, isolated, or repaired) played a significant role in behavior in the social interaction test in groups with both sexes (2-way ANOVA of group*sex on SI test PCs). This result is attributable to effects on PC2 ($F_{3,20} = 4.22$, $p = 0.0182$) with a significant effect of housing group and non-significant effect of sex (group: $p = 0.037$; sex $p = 0.08$; group*sex $p = 0.20$). PC2 is most influenced by autogrooming and allogrooming, and this effect appears to be driven most strongly by high levels of autogrooming in isolated females.

In contrast, neither sex nor housing had an effect on PCs derived from behaviors in the partner preference test (across groups with both sexes, or in analysis of females across all groups). Females across all groups exhibited similar, significant within-group preferences for huddling with the partner over the stranger (control: ($t_{26} = 4.24$, $p = 0.0003$), re-paired: ($t_{24} = 4.67$, $p < 0.0001$), group-living: ($t_{22} = 5.32$, $p < 0.0001$) (Fig. 2(**A**)). There were also no detectable sex differences in the single group (control) with both sexes, including in partner huddling ($t_{23.81} = 0.51$, $p = 0.61$), stranger huddling ($t_{23.65} = -0.60$, $p = 0.55$), and preference score (partner huddling/total huddling) ($t_{23.97} = 0.65$, $p = 0.52$).

### Partner preference was associated with decreased stranger interest across tests

Greater partner-directed affiliation in the partner preference test was associated with reduced stranger-directed affiliation in the social interaction test across all animals. Animals that spent more time in their partner's chamber in the PPT spent less time huddling with a stranger in the SI test ($r = -0.34$, $p = 0.022$) (in which

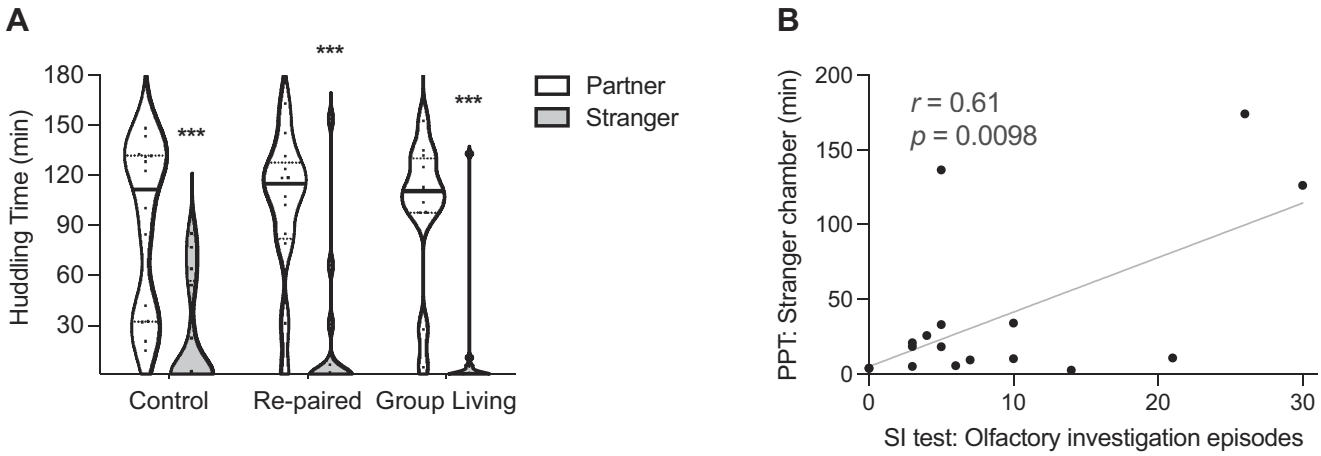


**Fig. 2. Social behavior.**(**A**) There were no differences in partner preference across females in all housing groups tested in the PPT (2-way ANOVA). Control (housed in stable pairs since weaning), re-paired for 2 weeks, and group-living females all displayed significant preferences for huddling with the partner over the stranger (*t*-test). (**B**) Stranger-directed olfactory investigation in the SI test was associated with stranger-directed behavior in the PPT, while increased partner interest in the PPT was associated with less investigation of the stranger in the SI test. ***$p < 0.005$, median indicated by solid line.

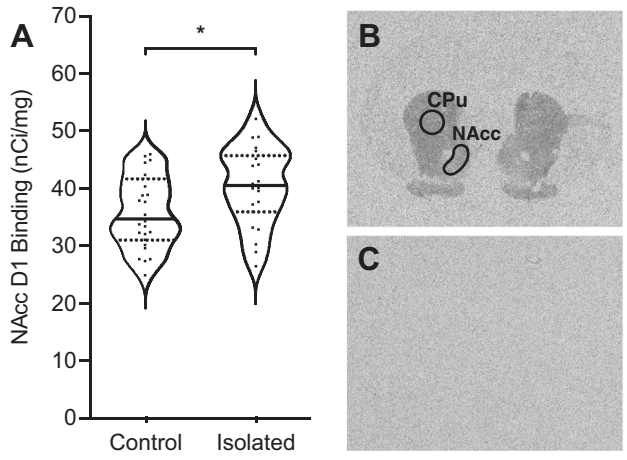


**Fig. 3.** (**A**) Dopamine receptor binding by housing: isolated animals displayed upregulated NAcc binding compared to control animals (*t*-test). (**B**) Sample autoradiogram illustrating binding in the nucleus accumbens (NAcc) and caudoputamen (CPu) with the tritiated ligand $^{3H}$SCH23390 with ketanserin. (**C**) Non-specific binding control: an adjacent brain section was treated with a selective D1 receptor antagonist SKF38393 together with the radioligand/ketanserin solution. *$p < 0.05$, median indicated by solid line.

only a stranger is present) and less time in olfactory investigation of a stranger ($r$ = -0.59, $p$ = 0.013). Similarly, animals with more bouts of partner huddling in the PPT spent less time huddling with a stranger in the SI test ($r$ = -0.33, $p$ = 0.025) and less time in olfactory investigation of a stranger ($r$ = -0.69, $p$ = 0.002). There was also a trend toward animals with higher partner huddling in the PPT displaying less olfactory investigation of strangers in the SI test ($r$ = -0.48, $p$ = 0.05).

Conversely, affiliation toward strangers in the PPT was associated with more prosocial behavior in the SI test. Time huddling with a stranger ($r$ = 0.56, $p$ = 0.021), time in a stranger's chamber ($r$ = 0.61, $p$ = 0.0098, Fig. 2(**B**)), and number of bouts of huddling with a stranger ($r$ = 0.56, $p$ = 0.021) in the PPT were positively correlated with time spent investigating a stranger in the SI test.

### Social environment was associated with variation in dopamine D1 receptor density

Dopamine D1 receptor density differed significantly by housing (Fig. 3). A MANOVA determined there was a main effect of housing, but not sex or interaction effects, on NAcc and CPu dopamine D1 receptor binding in groups with both sexes (control females, control males, isolated females, isolated males) ($F_{2,46}$ = 3.28, $p$ = 0.047). Isolated animals in groups with both sexes exhibited higher D1 receptor binding in the NAcc (Fig. 3 (**A**); $t_{50}$ = -2.49, $p$ = 0.016) and CPu ($t_{50}$ = -2.29, $p$ = 0.026) than control animals. A MANOVA across all female groups (control females, isolated females, re-paired females, and group-living females) did not identify a significant effect of housing on D1 receptor NAcc or CPu binding in only females ($F_{3,40}$ = 0.8755, $p$ = 0.46).

### Antisocial behavior in the partner preference test was correlated with dopamine D1 receptor density

Social behavior in the partner preference test significantly predicted D1 binding in the brain (MANOVA of PPT PCs on NAcc and CPu binding, effect of PC1 $p$ = 0.93, effect of PC2 $p$ = 0.017). PC2 ('sociability') was associated with decreased binding in both the NAcc ($p$ = 0.0067) and CPu ($p$ = 0.026). PC2 is most strongly influenced by time spent alone. Across all socially housed animals, both PC2 (Fig. 4(**A**); $r$ = 0.38, $p$ = 0.0062) and time alone (Fig. 4(**B**); $r$ = 0.37, $p$ = 0.0077) predicted D1 binding in the NAcc in linear associations. Higher D1 binding in the CPu ($r$ = 0.33, $p$ = 0.02) was also correlated with increased time spent alone.

Because housing (isolation) was associated with increased D1 receptor binding, we also assessed the relationship between D1 receptor binding and time spent alone within a single housing group (i.e., among voles housed in the same social environment). In group-living females, higher D1 binding in the NAcc was correlated with increased time spent alone ($r$ = 0.71, $p$ = 0.0094), but CPu binding was not ($r$ = 0.37, $p$ = 0.24). No significant correlations were found between NAcc dopamine D1 receptor density ($r$ = -0.095, $p$ = 0.51) or CPu dopamine D1 receptor density ($r$ = -0.13, $p$ = 0.37) and prosocial behaviors such as time huddling with the partner.

### Dopamine D1 receptor density was correlated between regions

Mean D1 binding differed by brain region (NAcc, CPu) across all animals ($t_{151.69}$ = -8.45, $p$ < 0.0001), but was strongly correlated between regions ($r$ = 0.65, $p$ < 0.0001).

## DISCUSSION

### Peer relationships differ from pair bonds

The current study assessed whether long-term, structural changes occur during the maintenance of new peer relationships—allowing for direct comparison to parallel work in prairie vole pair bonds—as well as the effects of prolonged social isolation and group housing. Unlike prairie vole males housed with new opposite-sex partners for two weeks (Aragona et al., 2006), dopamine D1 receptor density was not upregulated in prairie vole females housed with new same-sex partners for two weeks. This suggests that dopamine D1 receptor plasticity may not be involved in the maintenance of peer relationships. As in our prior studies of the role of dopamine neurotransmission in peer partner preferences in prairie voles (Lee and Beery, 2021) and in seasonally social meadow voles (Beery and Zucker, 2010), the mechanisms underlying peer relationships appear to be distinct from those underlying pair bonds and do not indicate reliance on altered dopamine signaling pathways.

D1 in prairie vole mate bonding is important for selective aggression toward strangers (Aragona and Wang, 2009), and this aggression is a crucial component of the maintenance of selective mate partnerships, especially in males (reviewed in Young et al., 2011). In the present study, we found no indication that selective aggression increased after novel same-sex pairing. If selective aggression were an important feature of prairie vole peer relationships, we should expect to see elevated aggression in the re-paired group (paired with new same-sex partners in adulthood), just as we would in prairie voles paired with new opposite-sex partners in adulthood. This lack of increase in aggression is consistent with the lack of increase in D1 upon pairing with a same-sex partner.

### D1 was associated with social isolation and reduced social behavior

Although we found no significant differences in dopamine D1 receptor density in re-paired females compared to control (long-term) pairs, D1 receptor binding was responsive to other social manipulations. In particular, socially isolated animals exhibited upregulated binding in the nucleus accumbens compared to control animals. This effect of social environment on dopamine receptor binding is a novel finding in prairie voles, but parallels work on social isolation in other rodent species. Social isolation, especially early social isolation, disrupts dopamine regulation in several rodent species. For example, early social deprivation in male and female socially monogamous mandarin voles (*Microtus mandarinus*) is associated with elevated dopamine, and increased mRNA expression of D1 receptors in the NAcc (Yu et al., 2013). Furthermore, early social deprivation inhibits partner preference formation in adult mandarin voles, with males and females spending more time exploring the cages of, and displaying aggression toward, unfamiliar animals—suggesting a relationship between upregulation of dopamine D1 receptors and reduced

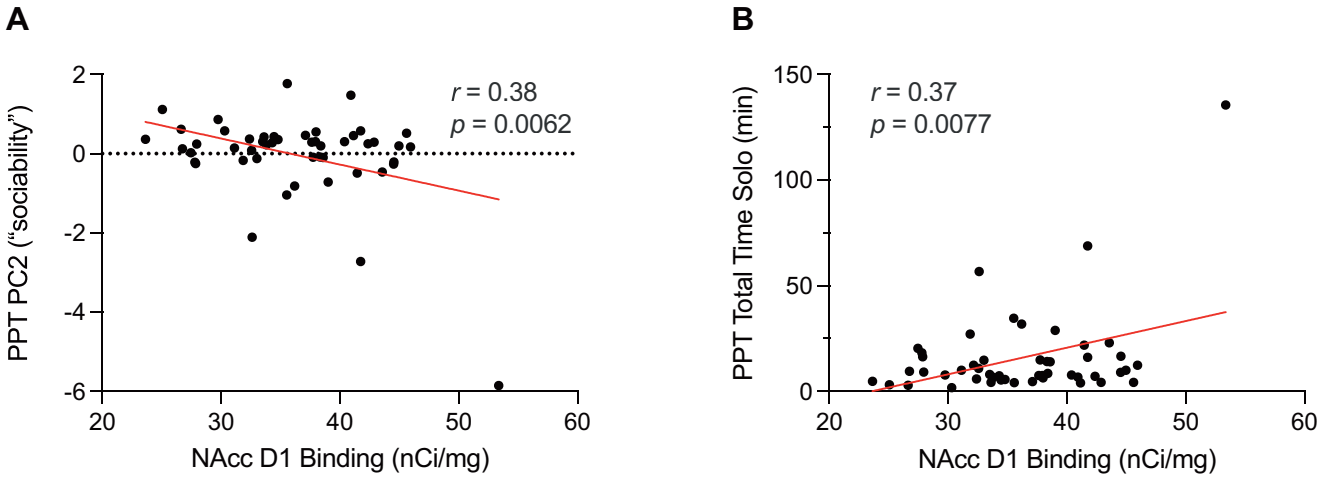


**Fig. 4.** D1 binding was correlated with social behavior across all animals tested in the PPT. (**A**) Principal component PC2 ('sociability') was negatively correlated with D1 binding. (**B**) Increased D1 binding in the NAcc was associated with more time spent alone in the PPT.

capacity for social bonding (Yu et al., 2013). Similarly, isolated mice exhibit increased D1 receptor density in the striatum compared to group-housed mice (Gariépy et al., 1995). This study furthermore suggests a role for dopamine D1 receptors in isolation-induced social reactivity, wherein high-aggression mice exhibit more attacks in social interaction tests when socially isolated, and low-aggression mice freeze (both asocial behaviors). These findings also parallel the correlations between D1 receptor density and reduced social behaviors found in the present study, most notably increased time alone.

As selectively social animals, prairie voles are rewarded by social partners and exhibit anxiety- and depression-like behaviors when socially isolated. It is possible that social isolation may impair an individual's ability to form partner preferences in the future, as in mandarin voles in Yu et al. (2013), or make an individual more reactive to social experiences, as in mice Gariépy et al. (1995). Since socially isolated animals could not be assessed for partner preference formation in the current study design, in the future it will be important to investigate the implications of upregulated dopamine D1 binding on future behavior. There is, however, existing evidence that early life social experiences, including social isolation, affect partner preference behavior later in life. For example, neonatal social isolation impairs adult partner preference behavior in female prairie voles (Barrett et al., 2015); males and females reared by single mothers rather than breeding pairs exhibit delayed partner preference formation (Ahern and Young, 2009); and handled females are more likely to form partner preferences in adulthood than control females (Bales et al., 2007). These studies suggest that social environment, and even a mild manipulation such as handling, can affect later pair bonding in profound ways.

In addition to finding that isolated voles exhibited greater D1 binding, we found that individual differences in D1 binding positively correlate with time spent alone in the PPT (even within voles housed in the same social environment). This association suggests that elevated striatal D1 density could be a cause as well as consequence of antisocial behavior. Relatedly, prairie voles exposed repeatedly to amphetamine exhibit both impaired pair bonding and elevated D1 receptor binding in the NAcc (Liu et al., 2010). Together these findings strongly suggest that higher D1 binding is reflective of impaired social ability.

While D1 binding in the NAcc was the focus of this study, we also measured CPu binding for comparison. Binding differed between these regions, but was highly correlated across subjects. In all cases in which effects related to NAcc binding density were found, effects in the CPu were either of lower magnitude (e.g. differences in D1 in isolated voles) or absent (e.g. the relationship between D1 binding and time spent alone), underscoring the relative importance of the NAcc.

### Social environment and behavior in the partner preference and social interaction tests

Partner preferences for familiar companions versus strangers were robustly displayed in control paired, re-paired, and group-living animals, as in Lee et al. (2019) and Lee and Beery (2021), wherein voles displayed strong peer partner preferences regardless of sex, day length, and pharmacological manipulation. This aligns with work on prairie vole mate partnerships, where opposite-sex partner preferences form readily in most conditions (Madrid et al., 2020). Partner preferences are also similar across conditions that produce differences in social reinforcement (Goodwin et al., 2019); for example, females and males express different patterns of social effort expended, but identical huddling preferences (Beery et al., 2021; Vahaba et al., 2022). Of particular interest, prairie voles housed in peer groups exhibited strong preferences for an arbitrarily selected group member 'partner' over an unfamiliar vole, as in group-housed meadow voles (Beery et al., 2009). This indicates that peer relationships in both species are *selective* but not *exclusive,* in contrast to prairie vole pair bonds. This may be related to the difference in the role of D1 in peer and mate relationships, with D1 particularly involved in the stranger aggression that helps maintain exclusivity. It would be interesting to test whether prairie voles readily form repeated new peer relationships, as in prairie vole pair bonds (Kenkel et al., 2019), and whether the strength of these new relationships is dependent on time since separation from the prior partner (Harbert et al., 2020).

There was a significant effect of housing environment on behavior in the social interaction test, driven by high levels of autogrooming in isolated females. Since autogrooming is self-directed and performed alone, this finding is consistent both with isolated animals exhibiting higher D1 binding and higher D1 binding positively correlating with more time spent alone in the partner preference test.

### Social behaviors were correlated across behavioral test types

Across all animals that underwent both PPT and SI tests, voles that spent more time near strangers in PPTs were more investigative and prosocial toward strangers in SI tests. Interestingly, partner affiliation in PPTs was associated with reduced prosocial interaction with strangers in the SI test—that is, more partner bonding relates to less investigation of novel voles even when the partner isn't present. This provides further evidence that, across social contexts, prairie vole peer relationships are highly selective, like pair bonds with mates. This behavioral consistency also provides evidence for the existence of animal personalities or behavioral syndromes in prairie voles (reviewed in Sih et al., 2004), and is consistent with prior findings in prairie voles within tests over time. For example, prairie voles exhibit similar partner preferences for their mate after 24 h and after 2 weeks of cohabitation (Vahaba et al., 2022). Similarly, partner huddling between prairie vole mates does not change when tested after 3 days or 10 days of cohabitation (Harbert et al., 2020).

Non-reproductive relationships between same-sex peers are a critical facet of the social lives of prairie voles as well as humans. While upregulation of dopamine D1 receptors corresponds with prairie vole

pair bond maintenance, this is not the case for maintenance of prairie vole peer relationships. This likely reflects the lack of relationship-induced aggression and exclusivity in peer relationships. However, it is probable that signaling pathways involved in other aspects of prairie vole pair bonds do play a role in peer relationships. For example, oxytocin is likely to shape selectivity of relationships in peer contexts, as it does in meadow voles (Beery and Zucker, 2010; Anacker et al., 2016). The present study also provides evidence of housing-mediated D1 plasticity in prairie vole peers, as well as effects of D1 on social avoidance, contributing to our understanding of the neural and behavioral consequences of social isolation in a selectively social species.

## ACKNOWLEDGEMENTS

We are grateful to Maddie Lerner, Selin Kubali, and Zoe Kruse for their help with behavioral testing and scoring. We would also like to thank the staff of the Smith College Animal Care Facility for animal care and colony maintenance. NL and AB conceived the study, NL and CK conducted the study and quantified results, NL and AB analyzed the data and wrote the manuscript, and all authors edited the manuscript.

### Funding

This work was supported by the National Institute of Mental Health of the National Institutes of Health under award R15MH113085.

## ETHICAL STATEMENT

We have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience.

## REFERENCES

Ahern T, Young L (2009) The impact of early life family structure on adult social attachment, alloparental behavior, and the neuropeptide systems regulating affiliative behaviors in the monogamous prairie vole (Microtus ochrogaster). Front Behav Neurosci 3. https://doi.org/10.3389/neuro.08.017.2009.

Anacker AMJ, Christensen JD, LaFlamme EM, Grunberg DM, Beery AK (2016) Septal oxytocin administration impairs peer affiliation via V1a receptors in female meadow voles. Psychoneuroendocrinology 68:156–162.

Aragona BJ, Wang Z (2009) Dopamine regulation of social choice in a monogamous rodent species. Front Behav Neurosci 3. https://doi.org/10.3389/neuro.08.015.2009/full.

Aragona BJ, Liu Y, Curtis JT, Stephan FK, Wang Z (2003) A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. J Neurosci 23:3483–3490.

Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, Wang Z (2006) Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci 9:133–139.

Bales KL, Lewis-Reese AD, Pfeifer LA, Kramer KM, Carter CS (2007) Early experience affects the traits of monogamy in a sexually dimorphic manner. Dev Psychobiol 49:335–342.

Barrett CE, Arambula SE, Young LJ (2015) The oxytocin system promotes resilience to the effects of neonatal isolation on adult social attachment in female prairie voles. Transl Psychiatry 5: e606–e.

Beery AK (2021a) Familiarity and Mate Preference Assessment with the Partner Preference Test. Curr Protoc 1:e173.

Beery AK, Zucker I (2010) Oxytocin and same-sex social behavior in female meadow voles. Neuroscience 169:665–673.

Beery AK, Routman DM, Zucker I (2009) Same-sex social behavior in meadow voles: Multiple and rapid formation of attachments. Physiol Behav 97:52–57.

Beery AK, Christensen JD, Lee NS, Blandino KL (2018) Specificity in Sociality: Mice and Prairie Voles Exhibit Different Patterns of Peer Affiliation. Front Behav Neurosci 12. https://doi.org/10.3389/fnbeh.2018.00050/full.

Beery AK, Lopez SA, Blandino KL, Lee NS, Bourdon NS (2021) Social selectivity and social motivation in voles. eLife 10:e72684.

Beery AK (2021b) Intervole Timer. Available from: https://github.com/BeeryLab/intervole_timer

Burkett JP, Andari E, Johnson ZV, Curry DC, de Waal FBM, Young LJ (2016) Oxytocin-dependent consolation behavior in rodents. Science 351:375–378.

Carter CS (2017) The Oxytocin-Vasopressin Pathway in the Context of Love and Fear. Front Endocrinol 8. https://doi.org/10.3389/fendo.2017.00356/full.

Dalton VS, Zavitsanou K (2011) Rapid Changes in D1 and D2 Dopamine Receptor Binding in Striatal Subregions after a Single Dose of Phencyclidine. Clin Psychopharmacol Neurosci 9:67–72.

DeVries AC, Johnson CL, Carter CS (1997) Familiarity and gender influence social preferences in prairie voles (Microtus ochrogaster). Can J Zool 75:295–301.

Friard O, Gamba M (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol 7:1325–1330.

Gallardo CM, Darvas M, Oviatt M, Chang CH, Michalik M, Huddy TF, Meyer EE, Shuster SA, Aguayo A, Hill EM, Kiani K, Ikpeazu J, Martinez JS, Purpura M, Smit AN, Patton DF, Mistlberger RE, Palmiter RD, Steele AD (2014) Dopamine receptor 1 neurons in the dorsal striatum regulate food anticipatory circadian activity rhythms in mice. eLife 3:e03781.

Gariépy J-L, Gendreau PL, Mailman RB, Tancer M, Lewis MH (1995) Rearing conditions alter social reactivity and D1 dopamine receptors in high- and low-aggressive mice. Pharmacol Biochem Behav 51:767–773.

Gingrich B, Liu Y, Cascio C, Wang Z, Insel TR (2000) Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (Microtus ochrogaster). Behav Neurosci 114:173–183.

Gobrogge K, Wang Z (2016) The ties that bond: neurochemistry of attachment in voles. Curr Opin Neurobiol 38:80–88.

Goodwin NL, Lopez SA, Lee NS, Beery AK (2019) Comparative role of reward in long-term peer and mate relationships in voles. Horm Behav 111:70–77.

Grippo AJ, Gerena D, Huang J, Kumar N, Shah M, Ughreja R, Sue CC (2007) Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles. Psychoneuroendocrinology 32:966–980.

Grippo AJ, Wu KD, Hassan I, Carter CS (2008) Social isolation in prairie voles induces behaviors relevant to negative affect: toward the development of a rodent model focused on co-occurring depression and anxiety. Depress Anxiety 25:E17–E26.

Harbert KJ, Pellegrini M, Gordon KM, Donaldson ZR (2020) How prior pair-bonding experience affects future bonding behavior in monogamous prairie voles. Animal Behav Cogn. https://doi.org/10.1101/2020.06.05.135160.

Harper SJ, Batzli GO (1997) Are staged dyadic encounters useful for studying aggressive behaviour of arvicoline rodents? Can J Zool 75:1051–1058.

Homberg JR, Olivier JDA, VandenBroeke M, Youn J, Ellenbroek AK, Karel P, Shan L, van Boxtel R, Ooms S, Balemans M, Langedijk J, Muller M, Vriend G, Cools AR, Cuppen E, Ellenbroek BA (2016) The role of the dopamine D1 receptor in social cognition: studies using a novel genetic rat model. Dis Model Mech 9:1147–1158.

Kenkel WM, Perkeybile AM, Yee JR, Carter CS (2019) Rewritable fidelity: How repeated pairings and age influence subsequent pair-bond formation in male prairie voles. Horm Behav 113:47–54.
Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, Timmermans PJ (2013) The resident-intruder paradigm: a standardized test for aggression, violence and social stress. JoVE:77.
Kravitz AV, Tye LD, Kreitzer AC (2012) Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816–818.
Lee NS, Beery AK (2021) The role of dopamine signaling in prairie vole peer relationships. Horm Behav 127 104876.
Lee NS, Goodwin NL, Freitas KE, Beery AK (2019) Affiliation, Aggression, and Selectivity of Peer Relationships in Meadow and Prairie Voles. Front Behav Neurosci 13. https://doi.org/10.3389/fnbeh.2019.00052/full.
LeSauter J, Balsam PD, Simpson EH, Silver R (2020) Overexpression of striatal D2 receptors reduces motivation thereby decreasing food anticipatory activity. Eur J Neurosci 51:71–81.
Lieberwirth C, Liu Y, Jia X, Wang Z (2012) Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. Horm Behav 62:357–366.
Liu Y, Aragona BJ, Young KA, Dietz DM, Kabbaj M, Mazei-Robison M, Nestler EJ, Wang Z (2010) Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. Proc Natl Acad Sci 107:1217–1222.
Liu Y, Wang ZX (2003) Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. Neuroscience 121:537–544.
Madrid JE, Parker KJ, Ophir AG (2020) Variation, plasticity, and alternative mating tactics: Revisiting what we know about the socially monogamous prairie vole. Adv Study Behav Vol. 52:203–242.
Mansour A, Meador-Woodruff J, Bunzow J, Civelli O, Akil H, Watson S (1990) Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization- receptor autoradiographic analysis. J Neurosci 10:2587–2600.
Mosher L, Godar S, Morissette M, Mcfarlin K, Scheggi S, Gambarana C, Fowler S, Paolo T, Bortolato M (2018) Steroid 5α-reductase 2 deficiency leads to reduced dominance-related and impulse-control behaviors. Psychoneuroendocrinology 91.
Numan M, Numan MJ, Pliakou N, Stolzenberg DS, Mullins OJ, Murphy JM, Smith CD (2005) The effects of D1 or D2 dopamine receptor antagonism in the medial preoptic area, ventral pallidum, or nucleus accumbens on the maternal retrieval response and other aspects of maternal behavior in rats. Behav Neurosci 119:1588–1604.
Parada M, King S, Li M, Fleming AS (2008) The roles of accumbal dopamine D1 and D2 receptors in maternal memory in rats. Behav Neurosci 122:368–376.
Resendez SL, Keyes PC, Day JJ, Hambro C, Austin CJ, Maina FK, Eidson LN, Porter-Stransky KA, Nevárez N, McLean JW (2016) Dopamine and opioid systems interact within the nucleus accumbens to maintain monogamous pair bonds. ELife 5. https://doi.org/10.7554/eLife.15325 e15325.
Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19:372–378.
Stolzenberg DS, McKenna JB, Keough S, Hancock R, Numan MJ, Numan M (2007) Dopamine $D_1$ receptor stimulation of the nucleus accumbens or the medial preoptic area promotes the onset of maternal behavior in pregnancy-terminated rats. Behav Neurosci 121:907–919.
Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007) D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 30:228–235.
Vahaba DM, Halstead ER, Donaldson ZR, Ahern TH, Beery AK (2022) Sex differences in the reward value of familiar mates in prairie voles. Genes Brain Behav 21:e12790.
Verharen JPH, Adan RAH, Vanderschuren LJMJ (2019) Differential contributions of striatal dopamine D1 and D2 receptors to component processes of value-based decision making. Neuropsychopharmacology 44:2195–2204.
Walum H, Young LJ (2018) The neural mechanisms and circuitry of the pair bond. Nat Rev Neurosci 1.
Wang Z, Yu G, Cascio C, Liu Y, Gingrich B, Insel TR (1999) Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (Microtus ochrogaster): a mechanism for pair bonding? Behav Neurosci 113:602.
Williams JR, Catania KC, Carter CS (1992) Development of partner preferences in female prairie voles (*Microtus ochrogaster*): the role of social and sexual experience. Horm Behav 26:339–349.
Yawata S, Yamaguchi T, Danjo T, Hikida T, Nakanishi S (2012) Pathway-specific control of reward learning and its flexibility via selective dopamine receptors in the nucleus accumbens. Proc Natl Acad Sci U S A 109:12764–12769.
Young KA, Gobrogge KL, Liu Y, Wang Z (2011) The neurobiology of pair bonding: Insights from a socially monogamous rodent. Front Neuroendocrinol 32:53–69. https://doi.org/10.1016/j.yfrne.2010.07.006.
Yu P, An S, Tai F, Wang J, Wu R, Wang B (2013) Early social deprivation impairs pair bonding and alters serum corticosterone and the NAcc dopamine system in mandarin voles. Psychoneuroendocrinology 38:3128–3138.

*(Received 3 September 2022, Accepted 5 February 2023)*
*(Available online 14 February 2023)*