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Physical separation from the mate diminishes male's attentiveness towards other females: a study in monogamous prairie voles *Microtus ochrogaster*

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Abstract

We tested whether continuous cohabitation in monogamous voles affects the mated male's attentiveness to his breeding partner versus another female. Each male was housed in a 3-chamber apparatus with a Focal female (FF) and a Control female (CF) for 13 days then placed in a T-maze to assess his attentiveness to and memory of those females. The Distal male remained physically separated from both females, but received their distal cues. The Separate male cohabited with the FF for 3 days then remained physically separated from both females. The Disrupt male's continuous cohabitation with the FF was disrupted by having him physically separated from her after 10 days and placed with the CF for the last 3 days. The Continuous male cohabited continuously with the FF for 13 days. With females in the T-maze, the Separate and Disrupt males spent more time near the FF's box and the Disrupt males spent more time manipulating the FF's box than the CF's box. The Separate males groomed themselves more when near the FF's box than the CF's box. The Distal and Continuous males' attentiveness to the two females did not differ. Results suggest that physical distance from the partner may reduce male's attentiveness toward other potential mates. Prairie voles might be similar to socially monogamous primates in using tactile cues as a signal for maintaining their social bonds.

Key words: attachment, monogamy, partner preference, partner separation, social cognition.

In mammals, attachment between mother and infants is common, but attachment between sexual partners is rare (Broad et al. 2006). Consequently, most mammalian males leave their partner after mating to reproduce with other females (Kleiman 1977). The mother, having bonded with the young, then raises them alone (Rosenblatt 2003; Numan and Insel 2003). Nonetheless, males in a few mammalian species continue to seek the proximity of their partner after mating (Dewsbury 1987; Reichard 2003). These males remain in physical contact with their mate, may produce multiple litters with her and are often found caring for the offspring (Hartung and Dewsbury 1979; Kleiman 1985; Lonstein and De Vries 2000). The evolutionary basis for the behavior of these so called monogamous males remains unclear. Rather than solving the mystery, recent studies using computer modeling of social behavior have reignited a debate as to why some males display a preference to remain with a single mate whereas others readily leave the natal nest in search of other females (Lukas and Clutton-Brock 2013; Opie et al. 2013).

While some researchers have sought answers by analyzing mammalian social behavior, others have focused on the brain to understand the mechanisms by which monogamy could have evolved. The latter line of research has led to the hypothesis that existing neural

networks for infant attachment evolved in response to species-specific selection pressures such that physical contact with a sexual partner became rewarding and separation from a sexual partner became distressful (Panksepp et al. 2012; Finkel and Eastwick 2015). Support for this hypothesis has come from animal models such as prairie voles that form a partner preference and display signs of depression when separated from their mate (Getz et al. 1981; Getz and Carter 1996; Sun et al. 2014). Although vole research has enhanced our understanding of how pair bonds are formed, it remains unclear how pair bonds may persist within a population under different environmental conditions to preserve the monogamous mating systems over time.

Laboratory studies have shown that male prairie voles begin protecting their pair bond from intruders shortly after copulation (Winslow et al. 1993; Insel et al. 1995). However, observations of voles in the field, or under seminatural conditions, have suggested that maintaining a stable pair bond in nature might be harder than in the laboratory. Several studies in freely interacting prairie voles have reported that sexual partners who cohabit do not always show exclusive parentage (Solomon et al. 2004; Ophir et al. 2008a). These studies have found evidence of extra-pair fertilizations among resident male–female pairs that share a home range (Ophir et al. 2008b). Therefore, it appears that while some male prairie voles mate exclusively with a single female at a time, others copulate outside the relationship.

Although genetic variability may mediate males' propensity to form a pair bond (Hammock et al. 2005; Barrett et al. 2013), field research on various prairie-vole populations has suggested that external ecological factors such as population density and availability of food and cover may override the male's inclinations for pair bonding and increase the likelihood that he engages in extra-pair fertilizations (Solomon et al. 2009; Mabry et al. 2011; Streatfeild et al. 2011). Given that the population density of prairie voles can increase from 10 to over 600 voles per hectare (Getz et al. 2001), it is plausible that the proximity of males to females in nature may impact the male's decision-making processes and shift his attention from his mate to other available females with which he has become familiar through shared space.

Previously, we attempted to simulate natural conditions of high population density in the laboratory by bringing the male prairie vole into proximity of multiple females then testing his attentiveness to his partner compared with another female with which he had no prior physical contact (Parker et al. 2011). As arvicoline rodents have been shown to have episodic-like memory of their preferred female (Ferkin et al. 2008), we also attempted to test the males' memory of the females' spatial location to determine if they could remember where they had encountered the female of their choice. Our data showed that males shift their attention from their partner to the other female when they were allowed to mate and cohabit with their partner for 72 h. However, we found no evidence that the males had remembered the spatial location of the females. It is possible that 72 h of cohabitation was not sufficient time for the males to form a memory of their partner's spatial location. Additionally, we found that males that mated and cohabited with their partner for 72 h were more likely to shift their attention to another female or her odor when that female was sexually receptive, but not when that female was sexually unreceptive (Rodriguez et al. 2013). However, the male in our previous studies had mated and cohabited with his partner for 72 h. Although this time period is long enough for bond formation, it is not sufficient for bond maintenance (Aragona et al. 2006). Therefore, our males might have shifted their attention from their partner to another female because the bond was not stable enough for them to reject other potential mates.

We designed the current study to test the hypothesis that continuous cohabitation with a female partner beyond the first 72 h influences the male's focus of attention and memory on his mate. We predicted that: 1) males with continuous post-mating cohabitation would attend to their partner and remember her spatial location rather than focusing their attention and memory on another female; 2) when cohabitation is disrupted through either separation of the mates or the physical presence of another female, the male would shift his attention and memory from his partner to the other female.

Materials and Methods

Subjects

Subjects were F3 generation of prairie voles that are maintained in our animal facility at Lehman College. The breeding colony was established in 2008 from the offspring of field animals that were originally captured in east-central Illinois. We minimize inbreeding in our colony by monitoring the relatedness of males and females that we select to pair for breeding. Prior to the experiment, all voles were weaned at 20 days, then housed with a same-sex sibling in ($48 \times 27 \times 20$ cm) clear plastic cages. As the gestation period of prairie voles is approximately 22 days and they mate postpartum (Hasler 1975; Witt et al. 1990), this procedure ensures that the first litter is weaned prior to the arrival of the next litter. The bottom of each vole's cage in our colony is covered with approximately 5 cm of bedding consisting of a layer of moistened peat moss covered by a second layer of wood shavings, and then filled with a top layer of straw. The bedding in all vole cages is discarded twice a week and replaced with fresh bedding. Water and food, consisting of sunflower seeds, rabbit chow, and cracked corn (Fisher and Son Inc., Somerville, NJ), are available *ad libitum*. All vole cages are kept in rooms with fluorescent lighting and at temperatures around 20–25 °C. The light: dark cycle for the rooms is set at 14:10 with lights on at 6:00 a.m. The experimental subjects were 48 adult sexually naïve males. For each male, 2 sexually naïve females, labeled as Focal (FF) and Control (FC), were used as stimulus. These females were unrelated to each other and to the experimental male; they were born to different parents and were from different litters. However, they were matched for age (born less than 10 days apart) and were of similar weights (± 10 g difference in body mass). All experimental and stimulus animals used in the study were 60–120 days old and were sexually inexperienced at the beginning of the experiment.

Procedures

We created two testing apparatuses: A) to simultaneously expose a male to distinct socio-sexual cues from 2 unrelated females in phase 1 of the experiment; B) to test the male's attentiveness to and memory of those females following his experience with them in phases 2 and 3 of the experiment, respectively. The male and the two females were exposed to each other in apparatus A (Figure 1A) for 13 days. We chose 13 days of housing based on previous research on prairie voles showing that while a pair bond is formed immediately after mating, 2 weeks of continuous cohabitation is required to maintain it (Aragona et al. 2006; Resendez and Aragona 2013). The apparatus was designed such that the male could be simultaneously exposed to the distal cues (visual, olfactory, and auditory) of the two females while preventing the two females from having physical

contact with each other. Bedding was provided, and food and water were available *ad libitum* in the 3 compartments of the cage. To ensure that the male received olfactory cues from both females, his bedding was discarded every other day and was replaced with a mixture of bedding soiled by the two females.

The design for phase 1 is depicted in Figure 1A. During the first 8 h in apparatus A, males in all groups were simultaneously exposed to distal cues of the 2 females then assigned to 4 groups ($N = 12$ per group). Next, a time-lapse video camera (Panasonic CCTV camera, model WV-BP330 Panasonic VCR, model AG-6540) was turned on and zoomed on apparatus A at a distance of 90 cm to verify the occurrence of mating. In the Distal group, the male was exposed only to the distal cues of both females and did not mate or cohabit with either one. The male in the Separate group was allowed to mate with the FF, but he was separated from her after mating and received the distal cues of both females for the remainder of his time in apparatus A. The Disrupt group was created to disrupt the pair bond by having the male mate and cohabit with the FF first then having him mate with the other female while continuing to receive the distal cues of his original mate. The Continuous group was set up to have the male mate with the FF and cohabit with her continuously while receiving the distal cues of the CF.

The design of the apparatus for phases 2 and 3 of the experiment is shown in Figure 1B. In phase 2, the male and the 2 females were placed together in apparatus B to test the male's attentiveness to the 2 females. To begin phase 2, the 2 females were removed from apparatus A and were placed in the plastic boxes and the opening to the boxes was covered with wire mesh. The placement of the boxes within the cages was randomized by assigning odd and even numbers to males so those with the odd number had the FF on their right, whereas those with the even number had the FF on their left. The male was placed in the third cage attached to the long arm of the habitrail tube with the tube opening covered to prevent him from entering the females' arena before the test began. Thus, apparatus B was designed such that the male had to choose alternate paths to reach one or the other female. Once the male was in one of the 2 cages, he could either explore the box containing a female at one end of the cage or explore the space at the other end of the cage.

After 5 min allowed for habituation, a digital video camera, zoomed on the apparatus, was turned on and the cover closing the male's access to the tube was removed. Immediately after the male had entered the tube, the cover was replaced to prevent him from returning to his start cage. The behavior of the male was then recorded for 10 min. We chose to study the males' behavior for 10 min

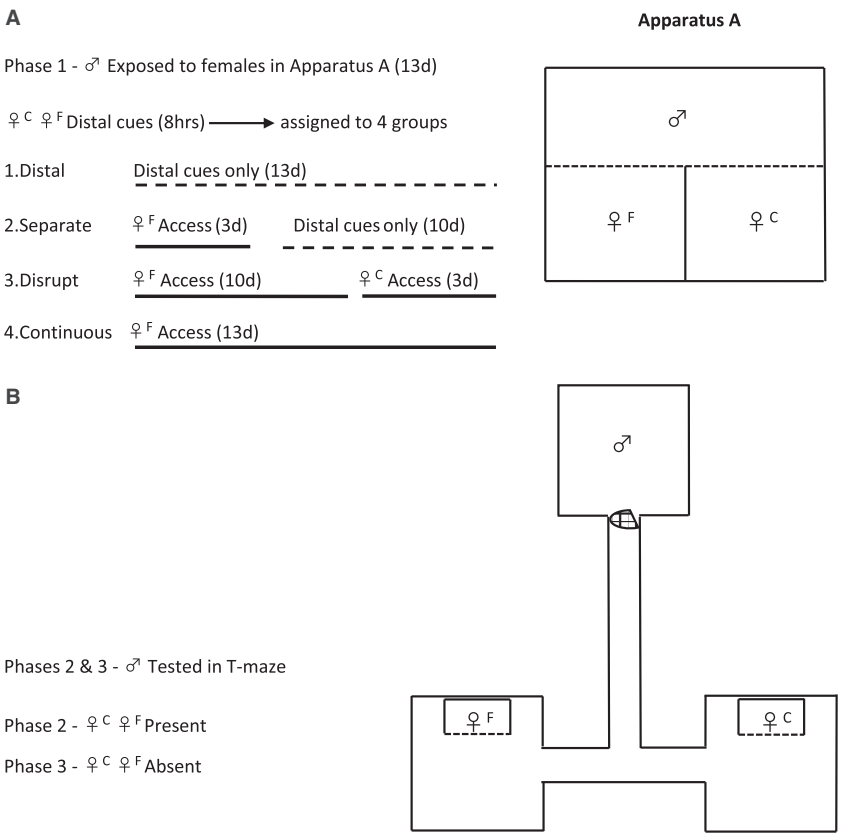

Figure 1. The experimental design and the behavioral testing apparatuses. (**A**) Apparatus A was designed to expose male prairie voles *Microtus ochrogaster* simultaneously to sensory cues of the Control (CF) and the Focal female (FF) in phase 1 of the experiment. The apparatus consisted of an oversized clear plastic cage (51 × 41 × 22 cm) divided into 2 halves with a perforated clear Plexiglas barrier. A solid aluminum sheet was placed at a right angle to the perforated barrier dividing one half of the cage into 2 smaller compartments of equal size (25.5 × 25.5 cm). The male was placed behind the perforated barrier while the 2 females were each placed in the 2 smaller compartments. The 2 females were separated from each other by a solid metal sheet to prevent them from visual and physical interactions. The male was separated from the 2 females by a perforated plastic barrier. (**B**) Apparatus B consisting of a T shaped habitrail tube (25 cm) connected to 3 small clear plastic cages (29 × 19 × 13 cm) was a modified version of that used in a previous study on meadow voles (Ferkin et al. 2008). The apparatus was designed to test male prairie vole's attentiveness to the CF and the FF or their cues in phase 2 and 3 of the experiment. Males' access to female cages was blocked before the tests began by covering the habitrail tube with a plastic cap. During phase 2, the male's behavior was observed when the females were randomly placed in boxes (15 × 10 × 8 cm) within a larger cage. During phase 3, male's behavior was observed when the females were removed from those boxes.

because we were interested in measuring their attentiveness to the boxes rather than their interactions with the females, and a previous study showed that 10 min is sufficient to assess cognition in voles (Ferkin et al. 2008). At the end of the test, the male and the 2 females were placed individually in separate cages for 60 min.

Immediately following the 60-min time period, each male was placed in apparatus B again to assess his behavior in phase 3 of the experiment. We chose to test the male's memory after 60 min based on a previous study in meadow voles showing that males can remember the previous location of their preferred female within 0.5–24 h of exposure to her (Ferkin et al. 2008). We tested the male's memory for the location of the 2 females by placing him alone in apparatus B so he could explore the boxes that previously contained the 2 females. Apparatus B and the 2 small boxes were washed to remove all odors, and they were placed in the same position as in phase 2. After 5 min, the camera, zoomed on apparatus B, was turned on and the cover closing the male's access to the tube was removed. The cover was replaced immediately after the male had left his cage to enter the tunnel, and his behavior was recorded in the empty apparatus for 10 min. The digital videotapes were later downloaded onto a computer and the male's behavior was analyzed using a behavioral analysis software program (ODlog Macropod Software) by a single observer that was blind to the experimental conditions.

All animals used in this study were cared for according to the guidelines for the use of animals in research published by the Association for the Study of Animal Behaviour and the Animal Behaviour Society (ASAB/ABS 2012). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Animal care and all procedures performed were in accordance with the ethical standards of Lehman College and were approved by the Lehman College Institutional Animal Care and Use Committee.

Behavioral analysis

The males' behaviors for phase 2 and phase 3 were analyzed separately. For each phase, the male's choice to turn right or left at the end of the T-shaped tube was recorded. The behavioral categories included: Attentiveness (sniffing, looking, and manipulating the front of the female's box); Engagement (time spent on top or behind the box, but not directly attending to the female); AttentiveEngagement (combined measures of Attentiveness and Engagement reflecting the duration of time the male manipulated each box); Self-Grooming; Activity (climbing, rearing, or walking); Total Time (time spent in each side of the apparatus on combined measures of Attention, Engagement, Self-Groom, and Activity); Exploration (time spent in tunnels outside of female cages).

Statistical analysis

When males were introduced into apparatus B in phase 2 and phase 3 of the experiment, they had a choice to either turn left or turn right in order to visit one or the other female. Based on the study by Ferkin et al. (2008), we used Sign tests to analyze the initial choice of males during phases 2 and 3 of the experiment. To test for group differences in Attentiveness, Engagement, Self-Grooming, and General Activity, we first created a new variable for each behavioral activity by deducting the duration of time males spent on the activity with the FF from the duration of time they spent on that activity with the CF. The new variables were tested for normality using the Shapiro–Wilk test and assessed for homogeneity of variance with the Levene's test. Depending on whether the variable had passed the tests or not, we used either a one-way ANOVA or the Kruskal–Wallis test to analyze the data. Significant group differences were further analyzed with Tukey post-hoc tests. In addition, for each group, we used either paired *t*-test or Wilcoxon signed rank test to determine if the duration of Total Time and AttentiveEngagement with the FF was different from that for the CF. Results are reported for significant differences at $P < 0.05$ and are stated for 2-tailed *t*-tests. Effect size is given by Cohen's *d* for *t*-tests and by *r* for Kruskal–Wallis and Wilcoxon signed-rank tests. For all statistical analysis, we used the Sigmastat statistical software program version 12.5.

Excluded subjects

Out of 36 males that were physically paired with the FF in the Separate, Continuous, and Disrupt groups ($N = 12$ in each group), 1 male in the Separate group did not copulate with the female during the first 3 days of cohabitation. That male and his female partners were excluded from the study and replaced by another set of male and female subjects. The occurrence of mating with the FF was verified in the remaining males. We were also able to verify the occurrence of mating with the CF for all 12 males in the Disrupt group. We detected no aggression (rearing, upright posture, chasing, or biting) between the Disrupt males and the CF, and observed all the pairs to mate within the first 24 h of being placed together.

Results

Males' initial choice in the T-maze

When males were first introduced into the apparatus, they immediately crossed the habitrail tube and chose to enter one or the other cage in which the box of the FF and CF were located. Results of the Sign tests showed that the male's initial choice to visit the FF and the CF during phase 2 when the females were present in apparatus B was not significant for the Distal (Sign test $P = 0.12$), Separate (Sign test $P = 0.4$), Disrupt (Sign test $P = 0.8$), and Continuous (Sign test $P = 0.8$) males. Out of the 12 males tested in each group, 6 in Distal, 8 in Separate, 5 in Disrupt, and 5 in Continuous groups chose to visit the CF first. We obtained similar results during phase 3 when the females were removed from the apparatus. The male's initial choice was not significant for the Distal (Sign test $P = 0.8$), Separate (Sign test $P = 0.2$), Disrupt (Sign test $P = 0.8$), and Continuous (Sign test $P = 0.8$) males. Out of the 12 males tested in each group, 5 in Distal, 3 in Separate, 5 in Disrupt, and 5 in Continuous groups chose to visit the CF first.

Males' behavior in the T-maze

The descriptive data for the duration (in seconds) of male's behavior in each group when FF and CF were present (phase 2) and when they were absent (phase 3) are shown in Table 1.

We had created a new variable to measure group differences in males' behavior by deducting the duration of time they spent on a given activity with the FF from the duration of time they spent on that activity with the CF. Analysis of the new variable showed that in phase 2, the Separate and the Disrupt males tended to spend more time attending to the box holding the FF than the box holding the CF. However, differences among the groups were not significant for Attentiveness (ANOVA test: $F_{3,\ 44} = 2.60$, $P = 0.06$). They were also not significant for Engagement (Kruskal–Wallis test: $\chi^2_3 = 1.61$, $P = 0.66$) or General Activity (ANOVA test: $F_{3,\ 44} = 2.24$, $P = 0.1$). We found a significant difference among the groups for the duration

Table 1. Male responses to females' boxes in their presence and in their absence

		Phase 2—present		Phase 3—absent	
		Female		Female	
Behavior[a]	Exposure	Focal $\bar{X}\pm SD$	Control $\bar{X}\pm$SD	Focal $\bar{X}\pm$SD	Control $\bar{X}\pm$SD
Attend	Distal	88.0 ± 56.4	96.6 ± 78.2	55.8 ± 62.1	64.6 ± 80.9
	Separate	101.9 ± 44.1	73.7 ± 23.1	52.8 ± 27.8	42.5 ± 18.7
	Disrupt	105.4 ± 56.4	56.4 ± 22.8	50.0 ± 29.6	76.7 ± 64.9
	Continuous	68.8 ± 37.9	97.5 ± 76.5	50.0 ± 29.6	76.7 ± 64.9
Engage	Distal	67.2 ± 39.1	93.9 ± 72.3	76.1 ± 116.6	69.7 ± 36.2
	Separate	86.4 ± 60.9	75.9 ± 37.4	67.7 ± 41.3	65.2 ± 36.0
	Disrupt	82.0 ± 33.1	74.7 ± 24.9	67.0 ± 40.3	45.1 ± 26.1
	Continuous	95.6 ± 55.8	76.9 ± 23.3	67.0 ± 40.3	45.1 ± 26.1
AttEng	Distal	155.2 ± 50.7	190.5 ± 81.4	131.9 ±123.7	134.3 ± 96.4
	Separate	188.3 ± 54.9	149.6 ± 50.4	120.5 ± 52.9	107.7 ± 44.4
	Disrupt	187.4 ± 64.5**	131.2 ± 34.2	117.0 ± 53.7	121.8 ± 70.5
	Continuous	164.4 ± 47.1	174.4 ± 65.1	117.0 ± 52.7	121.8 ± 70.5
Groom	Distal	13.1 ± 30.2	11.8 ± 11.3	10.6 ± 13.0	64.2 ± 113.3
	Separate	18.8 ± 32.0*	4.1 ± 7.2	15.9 ± 13.6	15.6 ± 19.0
	Disrupt	11.3 ± 21.0	5.7 ± 5.0	24.6 ± 46.4	11.9 ± 12.0
	Continuous	3.8 ± 4.6	10.5 ± 18.5	24.6 ± 46.4	11.9 ± 12.0
Active	Distal	65.0 ± 20.5	65.6 ± 23.6	44.9 ± 31.0	63.8 ± 48.2
	Separate	80.1 ± 35.2	60.0 ± 14.4	77.4 ± 61.6	88.5 ± 32.6
	Disrupt	60.8 ± 15.7	54.8 ± 15.2	58.6 ± 34.2	62.4 ± 31.5
	Continuous	59.0 ± 16.6	62.5 ± 18.4	58.6 ± 34.2	62.4 ± 31.5
Total	Distal	254.6 ± 84.6	285.1 ± 83.2	223.4 ±147.3	293.1 ± 145.7
	Separate	305.5 ± 63.7*	234.3 ± 48.0	241.2 ± 90.1	231.8 ± 79.0
	Disrupt	282.0 ± 61.8*	214.1 ± 39.1	218.1 ± 83.1	212.5 ± 103.7
	Continuous	247.2 ± 54.7	270.6 ± 78.0	218.1 ± 83.1	212.5 ± 103.7

[a] Duration measured in seconds., *and, **significant differences in male responses toward FF and CFs at $P < 0.05$ and $P < 0.01$, respectively (two-tailed *t*-tests).

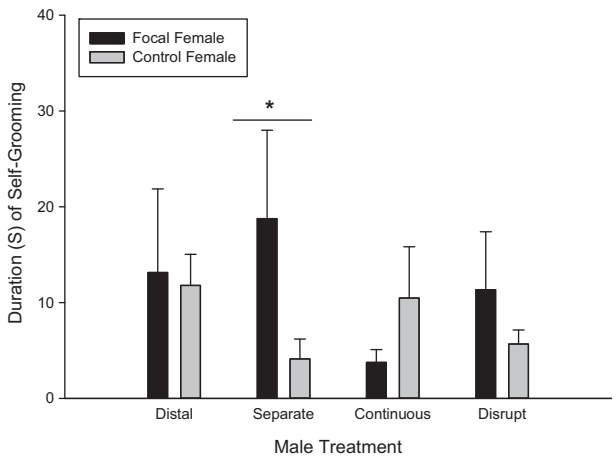

Figure 2. Self-grooming behavior. Duration of time in seconds ± SEM that males spent grooming themselves within the cages containing boxes of FF and CFs. In comparison to the Distal males, the Separate males spent more time grooming themselves within the cage holding the FF's box than within the cage holding the CF's box. The symbol * above the bars indicates significant differences in male responses towards Focal and CFs at $P < 0.05$.

of time males spent in Self-Grooming (Kruskal–Wallis test: $\chi^2_3 = 8.4$, $P = 0.04$). The Separate and Disrupt males spent more time grooming themselves when they were in the cage with the FF (Figure 2). Post-hoc tests showed that the difference in Self-Grooming between Separate and Distal males was statistically significant ($P < 0.05$).

For each group, we had also conducted paired *t*-tests to determine if the total time males spent in the cage of the FF and the CF and the time they spent manipulating their boxes differed. The differences were significant for the Separate group (Paired *t*-test: $t_{11} = 2.43$, $P = 0.03$, $d = 1.3$) and for the Disrupt group (Paired *t*-test: $t_{11} = 2.74$, $P = 0.02$, $d = 1.3$). Males in the Separate group spent more time in the cage containing the box of the FF than the CF (Figure 3A). Similarly, Males in the Disrupt group spent more time in the cage containing the box of the FF than the CF. Given that the time spent in Self-Grooming was significantly different among the groups, we reanalyzed the Total Time without Self-Grooming and found a significant difference for the Separate group (Paired *t*-test: $t_{11} = 2.2$, $P = 0.05$, $d = 1.0$) and for the Disrupt group (Paired *t*-test: $t_{11} = 2.69$, $P = 0.02$, $d = 1.3$). Additionally, we found a significant difference in AttentiveEngagement for the Disrupt males (Wilcoxon signed-rank test: $Z = -2.75$, $N = 12$, $P = 0.003$, $r = 0.8$). As shown in Table 1 and Figure 3B, these males spent more time attending to and engaged with the box (AttEng) holding the FF than the box holding the CF. The Separate males also spent more time with the box holding the FF than the box holding the CF, but the difference in their means showed a non-significant tendency (paired *t*-test: $t_{11} = 1.95$, $P = 0.08$).

When the females were removed from the boxes in phase 3 of the experiment, males' responses toward the boxes that previously held the FF and the CF did not differ (Table 1). Group comparisons of the new variable created to analyze males' behavior indicated no significant differences in Attentiveness (Kruskal–Wallis test: $\chi^2_3 = 2.63$, $P = 0.45$), Engagement (Kruskal–Wallis test: $\chi^2_3 = 6.04$, $P = 0.11$), Self-Grooming (Kruskal–Wallis test: $\chi^2_3 = 2.43$,

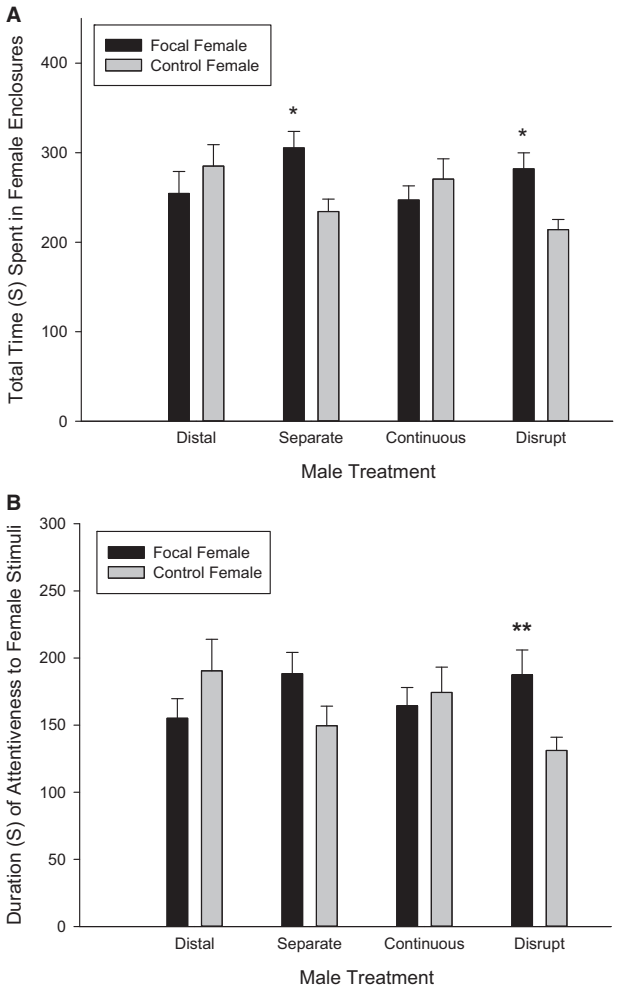

Figure 3. Male prairie voles *Microtus ochrogaster* tested in apparatus B for their attentiveness after 13 days of simultaneous exposure to Focal and CFs. **(A)** Duration of total time in seconds ± *SEM* that males were engaged in behavioral activities within the cages containing boxes of Focal and CFs. The Separate males and the Disrupt males spent more time engaged in all behavioral measures combined within the cage containing the FF's box. **(B)** Duration of time in seconds ± *SEM* that males spent looking at or manipulating the front of the boxes containing Focal and CFs. The Disrupt males attended to the box of the FF for a longer duration than the box of the CF. The symbols * and ** above the bars indicate significant differences in male responses towards Focal and CFs at $P < 0.05$ and $P < 0.01$ respectively.

$P = 0.49$), and General Activity (Kruskal–Wallis test: $\chi^2_3 = 1.65$, $P = 0.65$). Similarly, the results of paired *t*-tests showed that, within each group, there were no differences for AttentiveEngagement with the empty boxes or the Total Time spent in the cages when females were absent. However, comparison of Exploration (total time spent in the tunnels) between the two phases showed that males in all groups spend more time on Exploration (Mann–Whitney Rank Sum test: $W = 875.0$, $N = 48$, $P = 0.04$) in phase 3 when the females were absent than in phase 2 when the females were present.

Discussion

We tested whether continuous cohabitation between the male prairie vole and his mate would affect his decision to attend to her cues and remember her previous location or shift his attentiveness toward another potential mate. We had expected our Continuous males who had 13 days of physical contact with the FF to focus their attention on her rather than on the CF with which they had no physical contact. However, contrary to our expectations, the response of the Continuous males toward the cues of the FF and the CF did not differ. In contrast, our Separate males who were physically separated from the FF by a barrier for 10 days spent significantly more time near her than the CF with which they had no prior physical contact. Additionally, the Disrupt males who were also separated from the FF by a physical barrier spent significantly more time manipulating the box of the FF than the box of the CF. Previously, we had shown that following 72 h of mating and cohabitation with the FF, males pay more attention to the CF (Parker et al. 2011). However, in the current study, we found that the Separate males who had also mated and remained in physical contact with the FF for 72 h were more attentive to her than to the CF. We think that the difference in outcome between the 2 studies was the male's continued exposure to his mate's distal cues following the initial cohabitation period that we permitted in the present but not in the previous study. Having been exposed to the FF and then remaining physically separated from her may have shifted the male's preference for her. Based on these results, we hypothesize that physical separation from a mate induces a physiological change in the male that keeps him near her.

Past studies have shown that when the mate of a pair-bonded male prairie vole is removed for 4–5 days and the male is either housed alone or housed with another male, he displays physiological changes that are associated with stress. For example, the male shows passive-stress coping and depressive-like behaviors, autonomic imbalances, and increased blood corticosterone levels (Bosch et al. 2009; McNeal et al. 2013). Also, when the male's mate is removed for 4 weeks, he demonstrates anxiety-like behaviors and increased hypothalamic immunoreactivity for vasopressin, oxytocin, and corticotropin-releasing hormones (Sun et al. 2014). In our study, we did not remove the male's mate. Rather, we physically separated the bonded pair such that the male received auditory, olfactory, and visual cues but not tactile stimuli from his mate. It is plausible that by preventing the male from experiencing his mate's tactile cues, we increased his stress level and the heightened stress altered the focus of his attention. Our results suggest that the sense of touch might be an important factor in controlling male's focus of attention. Hence, one might presume that tactile cues under natural conditions may prevent the male from leaving his mate in search of other females, thus contributing to maintenance of the pair bond and persistence of social monogamy in nature.

There is field data to support the hypothesis that pair-bonded male prairie voles alter their behavior to remain close to their mate. Studies using radiotelementry to track prairie voles have shown that the areas used by pair-bonded males and females overlap to a great extent. The males use the same nesting burrow as the female and share a nest with her (Hofmann et al. 1984). There is also evidence that as the population density increases, these resident males modify their movement pattern and space themselves differently to guard their nest and minimize interactions with other social units (McGuire and Getz 1998). They decrease their home range size (Blondel et al. 2016), increase the number of runway systems, and remain confined within their own runways (Carroll and Getz 1976). This is in contrast to the behavior of non-resident males that wander to visit multiple social units and maintain much larger home ranges (Solomon and Jacquot 2002) that do not overlap with other females (Ophir et al. 2008b). The results of our study suggest that physical proximity, which facilitates exchange of tactile cues between the

mated pairs, may affect males' cognition thus altering his behavior so he limits his excursion out of his home range where he could encounter other females.

Although we did not test the males for anxiety or depression, we did measure the amount of time they spent grooming themselves in the cages holding the boxes of the FF and the CFs. Self-grooming in rodents has multiple functions (Spruijt et al. 1992; Ferkin and Leonard 2005) and can occur under comfort conditions (Kalueff and Tuohimaa 2004), but it is particularly sensitive to stressors (Kalueff and Tuohimaa 2005). Prairie voles use grooming to relieve stress not only in themselves, but also to console others. A recent study has shown that prairie voles groom their relative, but not an unfamiliar conspecific, that was exposed to stress (Burkett et al. 2016). We found that the Separate males who had experienced a lack of tactile cues from their mate for 10 days groomed themselves significantly more when they were near the box of the FF than the box of the CF. Although, the Disrupt males who had also experienced a lack of tactile cues from their mate groomed themselves more in the cage of the FF than the cage of the CF, the difference was not significant. This might be because the Disrupt males were separated from the FF for a shorter length of time or because their stress level was reduced after mating with the CF. Sexual activity is known to reduce stress in rodents (Waldherr and Neumann 2007), thus it is possible that mating with the CF buffered the Disrupt male's anxiety in response to physical separation from the FF.

We had set up the Disrupt group to determine if the male's motivation for extra-pair copulation would affect his selective attentiveness and memory. Having initially mated with the FF, there was a possibility that these males would treat the CF as an intruder and attack her when he was placed with her in apparatus A. Our conjecture was based on previous studies showing that male prairie voles develop aggression toward unfamiliar conspecifics of the opposite sex after mating (Getz et al. 1981; Winslow et al. 1993). However, we detected no aggression between the Disrupt males and the CF. We think that by exposing the Disrupt male and the CF to each other's distal cues, we might have familiarized them and thus increased the likelihood that they would mate rather than attack one another. When placed in the T-maze and given the choice to visit the two females, we had predicted that these males, having mated with both females would respond similarly to them. However, the Disrupt males showed the most robust response in favor of the FF with which they had cohabited for the initial 10 days compared with the CF with which they had cohabited for the final 3 days before being tested.

Although we found a preference for the FF's cues, we did not detect any evidence that cohabitation impacted the male's memory for the female's location. We had designed our experiment based on previous research that had shown meadow voles *Microtus pennsylvanicus*, without any prior training, could remember the spatial location of their preferred females (Ferkin et al. 2008). However, our present and past experiments have indicated that in absence of social cues male prairie voles do not show an interest in investigating empty containers and tunnels (Parker et al. 2011). Given that prairie voles are more likely to socially interact with their mating partner under most conditions than meadow voles (Madison 1980; Salo et al. 1993), they may rely on social rather than non-social cues to recall information (Matthews et al. 2013). Therefore, the type of memory test we used may work in meadow voles but not in prairie voles.

Another factor that may have affected the male's focus of attention is the reproductive status of the females to which he was exposed. We had verified that copulation had occurred more than once between each couple and all the mated pairs reproduced successfully after the experiment. A detailed study on the reproductive physiology of prairie voles has shown that sexually naïve females paired with an unfamiliar sexually naïve male for more than 12 h after the first copulation are certain to ovulate. The study also reported that the embryos enter the uterine horns 72 h after the first copulation and are implanted in the uterus a day later (Roberts et al. 1999). Thus, females in our study that were observed to mate had most likely ovulated and were pregnant. Further studies are required to determine if the pregnancy status of the mate would affect the male's attentiveness toward other familiar females in proximity.

The results of our study suggest that the male prairie vole's decision-making processes are guided by tactile cues he receives from his mate. Engaging in extra-pair copulations, which would require a temporary separation of the male prairie vole from his original pairmate may stress the male sufficiently to return him to the nest and prevent him from searching for other potential mates. Prairie voles may thus be similar to other socially monogamous primates in using touch as a cue for attending to their partner and maintaining their social bonds (Dunbar 2010; Campagnoli et al. 2015).

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