Published in final edited form as:

Anim Behav. 2013 November ; 86(5): . doi:10.1016/j.anbehav.2013.09.015.

Social recognition is context dependent in single male prairie voles

Da-Jiang Zheng¹, Lauren Foley, Asad Rehman, and Alexander G. Ophir*

Department of Zoology, Oklahoma State University, Stillwater, OK, U.S.A

Abstract

Single males might benefit from knowing the identity of neighbouring males when establishing and defending boundaries. Similarly, males should discriminate between individual females if this leads to more reproductive opportunities. Contextual social cues may alter the value of learning identity. Knowing the identity of competitors that intrude into an animal's territory may be more salient than knowing the identity of individuals on whose territory an animal is trespassing. Hence, social and environmental context could affect social recognition in many ways. Here we test social recognition of socially monogamous single male prairie voles, *Microtus ochrogaster*. In experiment 1 we tested recognition of male or female conspecifics and found that males discriminated between different males but not between different females. In experiment 2 we asked whether recognition of males is influenced when males are tested in their own cage (familiar), in a clean cage (neutral) or in the home cage of another male (unfamiliar). Although focal males discriminated between male conspecifics in all three contexts, individual variation in recognition was lower when males were tested in their home cage (in the presence of familiar social cues) compared to when the context lacked social cues (neutral). Experiment 1 indicates that selective pressures may have operated to enhance male territorial behaviour and indiscriminate mate selection. Experiment 2 suggests that the presence of a conspecific cue heightens social recognition and that home-field advantages might extend to social cognition. Taken together, our results indicate social recognition depends on the social and possibly territorial context.

Keywords

behavioural plasticity; context dependent; memory; *Microtus ochrogaster*; prairie vole; social recognition; territoriality

Social recognition can be broadly defined as the ability of an individual to distinguish one conspecific from another (Ferguson et al. 2002). This includes identification of an individual's specific social status, sex, or any other possible aspect of the phenotype. A more refined definition of this behaviour is how an individual integrates previous knowledge of another conspecific and changes their behaviour in a biased manner (Choleris et al. 2009). In either case, the importance of this behaviour should not be underappreciated given its

*Correspondence and present address: A. G. Ophir, 224 Uris Hall, Department of Psychology, Cornell University, Ithaca, NY 14853, U.S.A., ophir@cornell.edu (A. G. Ophir).

¹D.-J. Zheng is now at the Department of Integrative Biology, the University of Texas at Austin, 1 University Station #C0930, Austin, TX 78712, U.S.A., djzheng@utexas.edu (D.-J. Zheng).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

implications as a fundamental building block for more complex behavioural phenotypes such as aggression or social bonding.

Social recognition has been studied across many taxa (Gheusi et al. 1994; Tate et al. 2006; Bierbach et al. 2011; Jarcho et al. 2011; Sheehan & Tibbetts 2011; Bos & d'Ettorre 2012), but perhaps no animals have contributed to a deep understanding of social recognition more than rodents (Bielsky et al. 2005; Choleris et al. 2009; Albers 2012; Bychowski & Auger 2012; Wacker & Ludwig 2012). The paradigms developed in rodents that test social recognition are robust (Halpin 1974; Johnston 1993; Ferguson et al. 2002), often yielding reliable and predictable outcomes under controlled conditions (Macbeth et al. 2009a). Indeed, rodent models have greatly advanced our understanding of the mechanisms of social recognition and memory, and suggest that the neural mechanisms that govern social recognition are highly conserved (Ferguson et al. 2002; Choleris et al. 2009).

It should come as no surprise that animals frequently use social recognition in daily life. For instance, the dear enemy effect (Fisher 1954; Temeles 1994) implicitly assumes and relies upon an animal's ability to recognize and discriminate between conspecifics. Similarly, mate choice may rely on the ability to distinguish between potential partners, whether this functions as a means to avoid inbreeding with kin or to choose mates in good body condition (Kavaliers & Colwell 1995; Kavaliers et al. 2004; Mateo 2004; Zala et al. 2004). Unfortunately, seldom do studies designed to test social recognition in the laboratory consider the behavioural ecology of the study species (but see: Hurst et al. 1994; Ferkin & Johnston 1995; Kavaliers & Colwell 1995; Solomon & Rumbaugh 1997; Zala et al. 2004). Indeed, many studies explicitly designed to test social recognition ignore the social context or how it relates to this crucial cognitive ability. This is surprising because one of the prime motivations to understand social recognition is to gain a better appreciation for how this cognitive behaviour subserves other, more complex, social behaviours; this goal can only be achieved if an animal's natural history is taken into consideration.

Behaviours associated with a monogamous mating system represent one such area in which the role of social recognition is important in understanding a larger behavioural phenotype, and the biological relevance provides an important contextual backdrop. Several hypotheses attempting to explain the evolution of monogamy have been proposed, and the most prominent of these have highlighted the influence of male territorial behaviour used to defend ecological resources or mates from competing males (Orians 1969; Emlen & Oring 1977; Clutton-Brock & Vincent 1991; Wolff & Macdonald 2004). In the most general sense, monogamy can be characterized by selective affiliation with a mate, biparental care of offspring, and selective aggression involving territory and mate defence (Kleiman 1977; Clutton-Brock & Vincent 1991). Clearly, all three of these component behaviours require social recognition, but the sex and social context of the learning event is likely to produce differences in motivation and hold different biological valences for the individual. Nevertheless, it is unknown what role social recognition plays in each of these component behaviours.

Most laboratory studies that are designed to test social recognition have used mice or rats. When these studies have focused on male social recognition, stimulus animals will either be ovariectomized females (to control for effects of sexual receptivity) or juvenile males (to control for effects of male-male aggression). Although these methods have been effective at enabling researchers to assess the capacity for social recognition, they also have limited the ability to assess social recognition under social contexts that may carry particular biological relevance for this cognitive behaviour. With this in mind, prairie voles, *Microtus ochrogaster*, are a particularly well-suited species to investigate the influence of social recognition. Unlike rats and mice, female prairie vole oestrus is induced by exposure to

males or pheromones in their urine (Richmond & Stehn 1976; Carter et al. 1980; Dluzen et al. 1981). Thus, male motivation to investigate females for which sexual receptivity has not been induced should be comparable to that of rats and mice that are exposed to ovariectomized females without the need to perform surgery on stimulus females. Although field studies have indicated that territorial aggression probably results in exclusion of competitor males from established home ranges (Getz & Hofmann 1986; McGuire et al. 1990; Getz et al. 1997; Jacquot & Solomon 2004), prairie vole aggression in the laboratory is relatively low in males that are not pair bonded (Pitkow et al. 2001; Young & Wang 2004; Gobrogge et al. 2009). Thus, the likelihood of male–male aggression interfering with measures of social recognition when using single adult males as stimulus animals is low.

Furthermore, due to their socially monogamous mating system (Gavish et al. 1981; Carter & Getz 1993; Getz et al. 1993; Ophir et al. 2008), prairie voles offer an excellent opportunity to study social recognition in ways that relate to biologically meaningful contexts. Although the social organization of prairie voles is complex, all males begin life as nonreproductive unpaired individuals. At sexual maturity, males may choose to remain at the nest (as part of communal breeding units), or leave the nest (Getz & McGuire 1993; Getz et al. 1993). Males that leave the nest will form male–female breeding units, join other communal breeding units, or remain single and ‘wander’ (Solomon & Jacquot 2002; McGuire & Getz 2010; McGuire et al. 2013). Wanderers characteristically traverse large areas that they do not defend and are thought to mate opportunistically (Getz et al. 1993; Solomon & Jacquot 2002; Ophir et al. 2008). While some wanderers continue with this reproductive tactic until death, others will become ‘residents’ (McGuire & Getz 2010), a monogamous tactic in which males establish and defend territories, form pair bonds and exclude conspecifics, presumably to defend offspring and mate guard (Getz et al. 1993). Although residency is the most common tactic among prairie voles (estimated to account for 54–75% of the population: Getz et al. 1993; Solomon & Jacquot 2002; Ophir et al. 2008), a significant proportion of males will be sexually mature and single at some point in their lives. Indeed, about two-thirds of wanderers are former residents (from either male–female breeding units or communal breeding units) and transition to/from residency appears to be common (Solomon & Jacquot 2002; McGuire & Getz 2010; McGuire et al. 2013).

EXPERIMENT 1: DOES SOCIAL RECOGNITION VARY WITH SOCIAL CONTEXT?

The cognitive demands necessary to succeed during the single ‘wandering’ phase of life may differ based on the context in which an animal finds itself. For instance, natural selection may favour single males that are able to remember the location and identity of females in space and time as a means to maximizing reproductive opportunities (Gaulin & FitzGerald 1989; Jones et al. 2003; Ferkin et al. 2008; Ferkin 2011). Presumably, tracking female receptivity may lead to such benefits. Alternatively, the costs associated with learning female identity may provide little added benefit to reproduction, in which case it may not be worth the effort needed to discriminate between females. Thus, a lack of female recognition, which would lead to treating all females the same, may be equally or more beneficial to single males trying to maximize reproductive opportunities.

Recognition of other males may be important for single males if the value of establishing territories is high. For example, given that territorial residency is the most common reproductive tactic among prairie voles (Getz et al. 1993; Solomon & Jacquot 2002; Ophir et al. 2008), and transitions from single wanderer to paired resident are common, it seems plausible that single males would benefit by learning the identity of conspecifics with whom they will compete for territory. Alternatively, because single males are nonterritorial, encountering any male may present a threat to their reproductive success or survival.

regardless of their identity; in which case, recognition of other males may provide little value.

Our aim in this study was to assess the capacity for social recognition in single male prairie voles. We assumed that social recognition performance in different contexts would reflect the relevance and salience of social identity for single males. For example, the ability to discriminate between males should connote the importance of intermale aggression, whereas the ability to discriminate between females should suggest the reproductive value of female identity to males is relatively high. Here we test the general hypothesis that social recognition varies as a function of social context.

Methods

Subjects—All prairie voles in this experiment were F2 or F3 descendants from a breeding colony generously donated by Dr Tom Curtis (Oklahoma State University, Tulsa), and originally trapped from wild-caught voles from Champaign County, Illinois, U.S.A. We outbred these voles with wild prairie voles collected in Champaign County. New litters were weaned at 21 days old and housed in same-sex groups in polycarbonate cages ($29 \times 18 \times 13$ cm). We kept a 14:10 h light:dark cycle throughout the experiment (lights on at 0600 hours) and provided Rodent Chow 5000 (Harlan Teklad, Madison, WI, U.S.A) and water ad libitum. Males and females were separated on different racks placed on opposite sides of the colony room (approximately 3.5 m). All focal animals were housed with same-sex littermates and were 60–90 days of age when used in the behavioural trials described below. Our animal procedures were in accordance with the guidelines set and approved by the Institutional Animal Care and Use Committee of Oklahoma State University and in line with the ASAB/ABS Guidelines for the use of animals in research (ASAB/ABS 2012).

Twenty sexually naive adult males were selected to serve as focal males in a series of experiments designed to test whether single males recognize males, females, or both. Each focal male served in three tests (see below). Thus, experiment 1 represents a within-subject design.

Apparatus—We tested males in a custom-made apparatus designed to house animals and enable the testing of social recognition (Fig. 1a, b). The apparatus measured $20 \times 40 \times 28$ cm and was constructed of 1.27 cm thick transparent acrylic plastic. The apparatus was a rectangle with one doorway on each of the short walls and two side-by-side doorways on one of the long sides. The long side without doorways was taller than the other walls such that a mirror could be placed on the top of the apparatus resting at a 45° angle (Fig. 1b). This allowed us to simultaneously videorecord both a top-down and side view of an animal housed within the apparatus. Each doorway had guides that allowed us to either block the doorway (by sliding acrylic squares in front of the doorway), or slide a ‘presentation chamber’ into position. Presentation chambers were transparent acrylic boxes with hinged lids measuring 10.16 cm^3 . On one face of the presentation chamber boxes, we drilled 13 holes (1.27 cm diameter) to enable visual, auditory and olfactory contact. All doorways were closed using clear acrylic squares unless otherwise noted below.

Animals were housed in the main apparatus for 3 days prior to each of three tests (male habituation/dishabituation, female habituation/dishabituation and female social discrimination; see below). At least 2 days separated the end of one test and the beginning of the next. For the 3 days that animals lived in the apparatus, rodent chow and water were provided ad libitum and the floor was lined with Sani-chip bedding; food and water were removed immediately prior to behavioural testing. All tests were performed between 0800 and 1300 hours. Behavioural tests were recorded using a Sony SR-120 camcorder (Sony, New York City, NY, U.S.A.) in standard definition, which was placed 1 m away from the

tall (40 cm) face of the apparatus. Stimulus animals (see below) were approximately the same age ($-9.3 \text{ days} \pm 0.17$) as each other and the focal male. Behavioural data were always scored by an observer blind to the test conditions and identity of the animals serving in the experiments. The order of each test described below was counterbalanced across focal males.

Test A: habituation/dishabituation with male stimulus

We used the habituation/dishabituation test paradigm (Thor & Holloway 1981; Winslow & Camacho 1995) to test social recognition of males. To perform this test, we created a group of three animals consisting of the focal male, and two male stimulus animals. Each stimulus animal was unrelated to the focal male and to all other stimulus animals to which the focal male would be exposed (this also included stimulus animals in the tests described below).

To acclimate focal animals to the test apparatus, we placed them in the main apparatus for 30 min before initiating a trial; stimulus animals were placed in their respective presentation chambers for 30 min prior to being used in an experiment. To begin a trial we placed the focal animal in the centre of the apparatus and covered him using a white PVC cylinder (15.24 cm height, 7.62 cm in diameter). Next, we exposed each focal male to a stimulus male for 5 min, by replacing the acrylic square blocking the doorway with the presentation chamber containing a stimulus animal. The trial was initiated when we lifted the cylinder, freeing the focal male to interact with the stimulus male through the Plexiglas barrier.

During each presentation period, we measured the amount of time that focal males investigated the stimulus animal (defined as when a focal male's head was within 2 cm of the presentation chamber and he directed his nose towards the holes of the presentation chamber, presumably for the purposes of olfactory investigation) and the amount of time focal males attempted to contact the stimulus animal (defined as when focal males dug or chewed on the presentation chamber). We also quantified the frequency of aggressive interactions (defined as the number of times focal males bit or attempted to bite the stimulus animal through the holes), but this behaviour was extremely rare. Behavioural data were collected by watching a time-stamped digital recording and calculating the duration of each behavioural event (in seconds) using the timer and summing all events of the same type (e.g. investigation, attempted contact, etc.) across the trial. After 5 min, the presentation chamber was removed and the acrylic square was returned to close the doorway, initiating the beginning of a 15 min intertrial interval (iti). We repeated presentation of a stimulus animal in this way five times (Fig. 1c). The same stimulus animal was used for the first four presentations. On the fifth presentation, an unfamiliar stimulus male was presented to the focal male. During the acclimation period and intertrial intervals, presentation chambers holding the stimulus animals were moved at least 3 m away, and a cardboard partition blocked visual interaction.

We interpreted investigation time as reflecting focal males collecting social information about the stimulus animals via olfactory inspection, a measure of recognition. We interpreted attempted contact as reflecting focal males' motivation for social contact.

Test B: habituation/dishabituation with female stimulus

We used the same focal males in Test B that we had used in Test A. Test B was run in exactly the same way as Test A except that stimulus animals were sexually naïve mature females.

Test C: social discrimination test with female stimulus

Single males may be highly motivated to interact with females, regardless of whether they are able to recognize females individually. The nature of the habituation/dishabituation test limits focal animals (single males in this case) to interactions with one stimulus animal at a time. An undesired consequence of this design is that if males are sexually motivated to interact with females, then sustained interaction with stimulus females (even unreceptive ones) may resemble data indicating a lack of recognition. The social discrimination test controls for social motivation such as this by presenting two stimulus animals simultaneously during the habituation and dishabituation phases, and asking the focal animal to choose between a familiar and unfamiliar stimulus animal during the final presentation (Engelmann et al. 1995). The outcome of this test is a measure of social recognition that controls for sexual motivation.

We used the same focal males in Test C that we used in Tests A and B. The social discrimination test was conducted exactly as the female habituation/dishabituation test (Test B) with the following exceptions. First, two stimulus females were presented simultaneously at each end along the short walls. Second, on the fifth presentation, one of the two females was chosen at random, removed and replaced by a third stimulus female (see Fig. 1d). Finally, for this test, we quantified the total amount of time that males inspected or attempted to contact each of the two females during each presentation period.

Analysis

Habituation/dishabituation test—Previous studies focusing on rodent social recognition in habituation/dishabituation tests have shown that focal animals exhibit a robust and repeatable pattern of progressive decrease in olfactory inspection during ‘habituation’ trials (the first through the fourth presentation, P1–P4) followed by a return to high inspection in the ‘dishabituation’ trial (P5; see Fig. 1c) (Ferguson et al. 2002; Gheusi et al. 1994; Popik & Van Ree 1998). The reduction of social investigation in the first four presentations is interpreted as the focal animal’s habituation to an increasingly familiar social stimulus. Replacing the familiar social stimulus with an unfamiliar one tests social recognition. If the focal male recognizes that the new animal is different from the first, the focal male should increase its investigation of the novel social stimulus, usually to levels comparable to the first presentation of the original stimulus animal. When subjects do not increase their inspection of a novel stimulus, it is interpreted as the subjects’ inability to distinguish the first and second stimulus animals as different individuals (Ferguson et al. 2002).

We determined whether animals learned the social identity of conspecifics in the habituation/dishabituation test. First, we ran repeated measures ANOVA on log-transformed data to normalize variances across groups. We viewed the repeated presentation of stimulus animals as having two parts: a habituation phase (P1 to P3), and a dishabituation phase (P4 to P5). In experiment 1, we wanted to compare recognition of males to recognition of females. We therefore used two-factor repeated measures ANOVA using sex of stimulus animals (male or female) and presentation (either P1 through P3, or P4 to P5) as factors.

As a second measure to assess social recognition in the habituation/dishabituation test, we compared the difference between the time that focal males investigated stimulus animals presented in P4 with that of stimulus animals presented in P5. To control for total investigation time in both Tests A and B, we divided the differences between P5 and P4 by the sum of inspection time in these presentation periods. In this way we created a ‘social recognition score’ using the following equation: $((P5 - P4)/(P5 + P4))$, which could be used to compare recognition in the social discrimination test (see below).

Social discrimination test—In the social discrimination test, a progressive drop in investigation time of the two stimulus animals presented repeatedly (P1–P4) would be expected (Fig. 1d). However, the critical measure in this test was to determine whether focal animals would spend more time investigating an unfamiliar animal relative to a familiar animal during the final presentation (P5). Recognition is determined in the final presentation if a subject investigates a novel individual more than a familiar individual when both are presented simultaneously (Fig. 1d). Therefore, in Test C, we used a one-tailed paired *t* test to compare the time that focal males spent investigating novel and familiar females during P5. We used a one-tailed test because of the a priori expectation that, if recognition occurs at all, focal males should investigate the novel individual more than the familiar one. Previous experiments with rodents support this approach (Macbeth et al. 2009a, b).

As for Tests A and B, we calculated a ‘social recognition score’ by taking the difference between the times that the focal male spent with the novel and familiar females at test, divided by the sum of the male’s total investigation during the final choice. This allowed us to compare social recognition between the habituation/dishabituation tests and the social discrimination test, despite the differences in procedure.

Results and Discussion

One focal male unexpectedly died before serving in all three treatments; therefore, our sample size was $N = 19$ focal males. Our results from the habituation/dishabituation tests (Tests A and B) indicated that focal males habituated to the presentation of stimulus animals (two-factor ANOVA: $F_{2,72} = 7.03$, $P = 0.002$) and habituated to males and females equally ($F_{1,36} = 0.06$, $P = 0.82$; see Fig. 2a, left panel), with no significant interaction between these factors ($F_{2,72} = 1.32$, $P = 0.27$). We assessed social motivation by quantifying the time that the focal male spent attempting to contact the stimulus animals (see Methods). Across the habituation phase, we found no main effect of attempted contact across presentation ($F_{2,72} = 0.66$, $P = 0.52$) or sex of the stimulus ($F_{1,36} = 0.44$, $P = 0.51$), and we found no interaction between presentation and stimulus sex ($F_{2,72} = 1.24$, $P = 0.29$; Fig. 2b, left panel).

Next we determined whether focal males discriminated between the identity of novel and familiar stimulus animals by comparing the final presentation with the familiar stimulus animal (P4) and the presentation of the novel stimulus animal (P5). A main effect of presentation ($F_{1,36} = 10.56$, $P = 0.003$) indicated that focal males increased their inspection time in the dishabituation trial. Although no main effect of sex was apparent ($F_{1,36} = 0.18$, $P = 0.68$), an interaction between presentation (P4 versus P5) and sex (male versus female) indicated that focal males discriminated between males, but not between females ($F_{1,36} = 8.57$, $P = 0.006$; Fig. 2a, right panel). In contrast, we found no significant differences in attempted contact (a proxy of social motivation) during the dishabituation phase (P4–P5) for presentation ($F_{1,36} = 0.02$, $P = 0.89$) or sex ($F_{1,36} = 0.08$, $P = 0.77$), and we found no interaction effect between these factors ($F_{1,36} = 1.49$, $P = 0.23$; Fig. 2b, right panel).

The results of the habituation/dishabituation test using females (Test B) indicated that males did not discriminate between females. Fortunately, we performed a second comparison of female recognition by males (social discrimination test, Test C) to control for effects of sexual motivation on the part of focal males. The second test of social recognition with females had the added benefit of ensuring that the males’ lack of female recognition was robust across different testing conditions. Focal males did not differ in the time they inspected the novel and familiar females in the social discrimination test on the test presentation (P5; one-tailed paired *t* test: $t_{18} = 1.61$, $P = 0.06$; Fig. 3a). Although the difference in time that males investigated novel and familiar females was not statistically significant, the one-tailed test indicated that males showed a nonsignificant tendency to spend more time with the novel female. However, despite the a priori justification for a one-

tailed test, results from the more conservative two-tailed test ($P = 0.13$) strengthened our conclusion that males did not discriminate between females. Males showed no difference in the amount of time they attempted to contact novel females and familiar females in the social discrimination test ($t_{18} = 1.21$, one-tailed $P = 0.24$; Fig. 3b).

Finally, we calculated a 'social recognition score' for males in each social context (male social recognition, female social recognition and female social discrimination). These results were consistent with those discussed above; focal males showed recognition of other males but not of females (Fig. 4). This conclusion is supported by the fact that the recognition score across the three conditions was significantly different (one-factor ANOVA: $F_{2,36} = 3.21$, $P = 0.05$). Post hoc tests revealed that focal males showed significantly more recognition of other males than they did of females in the habituation/dishabituation tests (Tukey–Kramer test; $P = 0.009$), but recognition did not differ between other conditions. Furthermore, the recognition score should have been 0 if recognition was impaired. We therefore conducted one-sample t tests to ask whether focal males' recognition scores differed from 0 (i.e. if they demonstrated social recognition) in each condition. Although male recognition of other males significantly differed from 0 (one-sample t test: $t_{18} = 5.06$, $P < 0.0001$), recognition of females in the habituation/dishabituation test ($t_{18} = 0.13$, $P = 0.90$) and in the social discrimination test ($t_{18} = 0.60$, $P = 0.56$) did not differ significantly from 0, indicating a lack of recognition when males were exposed to females but not when they were exposed to other males.

EXPERIMENT 2: DOES TERRITORIAL CONTEXT INFLUENCE MALE SOCIAL RECOGNITION?

The results from experiment 1 indicate that social context has the potential to influence individual recognition, and it implies that learning male identity is potentially valuable to single male prairie voles. However, the conditions under which this information is most relevant are unclear. Most male prairie voles are territorial residents. Social recognition of other males might aid males, if knowing the identity of other male conspecifics facilitates patrolling, defending and possibly establishing territories. Alternatively, most male prairie voles will be wanderers at some point in their lives³ (McGuire & Getz 2010), and territorial residents are known to engage in extrapair copulations (Ophir et al. 2008; Streatfeild et al. 2011). Thus, learning the identity of male conspecifics when a male is intruding into an established territory of another male could prove beneficial. For instance, given that male–male social interactions that occur on territories of other males are most likely to precede an agonistic interaction, learning male identity when on foreign ground may serve as a highly salient social signal that would reinforce identity learning, in turn helping to mitigate the probability of incurring bodily harm imposed by aggressive territorial males. In either case, we expect that the local context of whether a male is in familiar or unfamiliar territory might affect his ability to recognize other males. Here we test the influence of territorial context on the social recognition abilities of sexually naïve single male prairie voles.

Methods

Subjects—We selected 37 sexually naïve adult males (age 60–120 days old) to serve in an experiment designed to test whether single males recognize other males when tested in a socially familiar, unfamiliar or neutral context. All animals used in experiment 2 differed from those serving in experiment 1. Males served in only one group (between-subjects design), and stimulus and focal males were of similar age (5.7 ± 1.04 days). The same behavioural apparatus described above was used in experiment 2. The procedure for exposing stimulus males to focal males in experiment 2 was the same as the methods described for the habituation/dishabituation test with male stimulus animals (Fig. 1c).

Experiment 2 differed from experiment 1 (Test A) in the length of time males lived in the testing apparatus and whether they were tested in the apparatus in which they were housed (see below).

Territorial context trials—Focal males were housed in a test apparatus for 7 days to establish a home cage. To initiate testing, at the end of 7 days, focal males were (A) removed and immediately returned to their own home test apparatus (familiar, $N = 12$), (B) removed from a home test apparatus and placed in the home apparatus of another male that had occupied the cage for 7 days (unfamiliar, $N = 13$), or (C) removed from a home test apparatus and placed in a test apparatus with clean bedding (neutral, $N = 12$). Therefore, 7-day-old soiled bedding from either the focal male or an unfamiliar male remained in the test cages in ‘familiar’ and ‘unfamiliar’ conditions, respectively. We used the cages housing focal males assigned the neutral or unfamiliar conditions as test arenas for focal males assigned to the unfamiliar condition. This ensured that the unfamiliar soiled bedding did not belong to the stimulus animals, and therefore did not provide odour cues that would help focal males in the unfamiliar condition identify the stimulus males. The bedding was fresh and unsoiled in the ‘neutral’ condition.

Analysis—As in experiment 1, we determined whether males learned the social identity of other male conspecifics in the habituation/dishabituation test by using a repeated measures ANOVA on log-transformed data to meet the assumptions necessary for parametric analyses. Our factors were territorial context (familiar, unfamiliar, or neutral) and presentation (P1–P3 or P4–P5). We also compared the ‘social recognition scores’; that is, the difference in time that focal males investigated stimulus males, controlling for total investigation time $((P4 - P5) / (P4 + P5))$ across each territorial context.

Results and Discussion

Territorial context did not affect social recognition across the three conditions we tested: familiar, unfamiliar or neutral. Although focal males habituated to stimulus males in the habituation phase ($F_{2,68} = 20.83$, $P < 0.0001$; Fig. 5a, left panel), habituation did not differ across territorial contexts ($F_{2,34} = 1.32$, $P = 0.28$), and there was no interaction between factors ($F_{4,68} = 0.82$, $P = 0.52$). Similarly, we found a main effect for males in the social recognition test to dishabituate between P4 and P5 ($F_{1,33} = 6.24$, $P = 0.02$; Fig. 5a, right panel), but territorial context had no effect on dishabituation ($F_{2,33} = 1.30$, $P = 0.29$), and we found no interaction between context and presentation ($F_{2,33} = 1.14$, $P = 0.33$).

Attempted contact did not differ across presentation (habituation: $F_{2,68} = 0.51$, $P = 0.60$; dishabituation: $F_{1,34} < 0.0001$, $P = 0.99$) or between contexts (habituation: $F_{2,34} = 0.003$, $P = 0.99$; dishabituation: $F_{2,34} = 1.20$, $P = 0.31$), and there was no interaction between these factors (habituation: $F_{4,68} = 1.04$, $P = 0.39$; dishabituation: $F_{2,34} = 0.01$, $P = 0.99$) in either the habituation phase (P1–P3) or the dishabituation phase (P4 versus P5).

We found no main effect for social recognition scores across the three contexts (one-factor ANOVA: $F_{2,36} = 1.27$, $P = 0.29$; Fig. 5b), further suggesting that territorial context does not disrupt social recognition of other males. In contrast, we found that only the recognition scores of males tested under familiar (home cage) conditions significantly differed from 0, indicating that recognition was strongest when tested under these conditions (one-sample t test: $t_{11} = 3.48$, $P < 0.01$). The recognition scores for males tested in an unfamiliar context were marginally different from zero ($t_{12} = 2.09$, $P = 0.058$), while recognition scores for males tested under the neutral context did not differ from 0 ($t_{11} = 0.77$, $P = 0.47$). Taken together, these contradictory findings imply that while males appeared to demonstrate social recognition in all three contexts, the degree to which they demonstrated social recognition

may have been weakly diminished in settings that smelled unfamiliar and/or that lacked social markings altogether.

On closer examination of the data, we noticed that individual variation within each territorial context was qualitatively different (see Fig. 6). Individual variation in olfactory inspection in the home cage (familiar) across presentations (P1–P5) was noticeably lower compared to the unfamiliar context (simulating an intrusion), and both appeared qualitatively distinct from the neutral context. To determine whether the individual variation within each group (familiar, unfamiliar and neutral) was statistically different across groups (Table 1), we analysed our untransformed data with Mauchly's test of sphericity. We found that the variances were indeed significantly different ($W = 0.43$, $\chi^2_9 = 30.18$, $P < 0.0001$; Fig. 6, Table 1). These results support the hypothesis that while most individuals are particularly good at social recognition learning and discrimination when placed in a context that is familiar, an environment other than a home cage (particularly one lacking social cues such as odour) may interfere with the ability to distinguish between conspecifics for some individuals. Taken together, our results suggest that the process of learning social identity is robust on familiar territory and comparably variable when social scents are absent or indicate that a competing male defends the territory.

GENERAL DISCUSSION

We have demonstrated that social context influences the degree to which males recognize conspecifics. Specifically, the sex of the stimulus animal is important in determining whether males distinguish between new and familiar conspecifics. In contrast, the olfactory cues commonly associated with territorial context appear to have little effect on the overall ability of males to recognize other males. However, we note the peculiar influence of these social cues on individual performance during recognition tests; either the absence of social cues (neutral context) appears to increase the individual variation in social recognition ability, or the presence of an individual's own scent (home cage context) appears to reduce the individual variation in social recognition ability.

Sex-specific Recognition of Males

Our results indicate that males show clear social recognition of other males but not of females. The attention to male, but not female, identity is consistent with the hypothesis that distinguishing among other males may be adaptive for single males. However, our experiments are unable to determine what form any such benefit might take, and several hypotheses could be generated.

Because most male prairie voles are paired 'residents' (Getz et al. 1993; Solomon & Jacquot 2002; Ophir et al. 2008) and residency appears to be associated with territoriality (Getz & Hofmann 1986; McGuire et al. 1990; Getz et al. 1997; Jacquot & Solomon 2004), we prefer the hypothesis that social recognition might enhance reproductive success through male–male competition for territory. This interpretation implies that knowing the identity of male neighbours is important to males, and it may enhance their ability to establish and possibly defend territories from known conspecifics. This hypothesis assumes that establishing home territories precedes establishing pair bonds. However, it is unknown whether territories are first established by adult males, females or both, and it is unknown how prairie voles first establish territories. Nevertheless, male resident prairie voles appear to exclude nonresident conspecifics (Getz & Hofmann 1986; McGuire et al. 1990; Getz et al. 1997; Jacquot & Solomon 2004). For example, fewer nonresident males are captured near the territories of social groups containing resident adult males, suggesting that resident males may attempt to exclude nonresidents (McGuire et al. 1990). This behaviour may be responsible for producing the stereotypical home range patterns in which breeding pairs show a high degree

of overlap with each other but low overlapping home ranges with other breeding pairs (Getz & Hofmann 1986; Ophir et al. 2008; Ostfeld 1990).

The special attention that males dedicate to learning male identity and the strengthening effect that a familiar home environment has on social recognition for other males support the idea that recognition is important for territory defence and male–male agonistic interactions. The ability to recognize possible rivals is particularly important when familiarity of such rivals enables males to reduce the potential for injury by reducing the intensity of territorial conflict (Ydenberg et al. 1988). The ‘dear enemy’ hypothesis proposes that reduced aggression towards familiar rivals is predicated upon previous agonistic interactions with those animals and the memory for neighbouring rivals is subserved by those aggressive interactions. More recently, it has been suggested that aggressive interactions are unnecessary for maintaining memory of neighbours and the reduced aggression that results (delBarco-Trillo et al. 2009). Although our study could not test the aggressive behaviour for familiar or unfamiliar males because aggression was so rare, our results are consistent with this suggestion that social recognition of potential rivals does not require agonistic behaviour.

Sex-specific Recognition of Females

Compared to their ability to recognize other males, our results suggest that single males are unable to, or uninterested in, recognizing individual females (Fig. 2a). If males are indeed indiscriminating, then these results may explain why male prairie voles in the field opportunistically form a pair with the first available mate (Getz et al. 2004). Although the opportunity for a choice during the social discrimination task tended to enhance investigation of unfamiliar females (Fig. 3a), this difference was not statistically significant. Our conclusion that males do not discriminate between females is further strengthened by our results showing that recognition scores for females in both the habituation/dishabituation and social discrimination tests were not significantly different from zero.

Traditionally, social recognition tests are based on the assumption that animals investigate familiar conspecifics less (hence the progressive reduction of investigation over a series of exposures) and that unfamiliar conspecifics will elicit high levels of investigation. Therefore, a lack of so-called ‘dishabituation’ is interpreted as evidence for social recognition impairment (Ferguson et al. 2002). Single males in our study did not dishabituate to the presentation of novel females, indicating that either they did not distinguish between the familiar and novel females, or they were no longer interested in investigating females. Because male assessment of conspecifics was restricted by a partition, we cannot rule out that physical contact might have enhanced social recognition by increasing the salience of the stimulus animals. However, we note that lack of physical contact has been used successfully in other tests of social recognition in several rodent species (e.g. Halpin 1974; Newman & Halpin 1988; Tang-Martínez et al. 1993); this study, experiment 1, Test A). Nevertheless, the salience of the stimulus can influence whether or not animals demonstrate dishabituation. For example, social recognition in mice is a short-lived phenomenon, often lasting no more than a couple of hours. However, in some contexts, such as when a stimulus mouse is infected with parasites, social recognition lasts up to 24 h (Kavaliers & Colwell 1995; Kavaliers et al. 2004).

Ultimately, it will be extremely difficult to determine whether the motivation for recognition or the ability for recognition accounts for results such as ours. While the males’ sustained attempted contact with females suggests that social motivation did not wane (Fig. 3), it is certainly possible that single males have the capacity for female social recognition, but they are simply nondiscriminating when it comes to evaluating and selecting potential mates. In this case, it may not matter who the mate is, as long as a mate is secured; thus, the effort

needed for social recognition of females may hold little value for single males. Sexual selection theory would support this general notion that males should choose mates indiscriminately (Bateman 1948; Emlen & Oring 1977). Although male mate choice is relatively less studied compared with that of females (in general, and specifically among prairie voles), some evidence exists that male prairie voles select females based on their reproductive output with female partners (Curtis 2010). In light of the socially monogamous mating system that prairie voles demonstrate (Gavish et al. 1981; Carter & Getz 1993; Getz et al. 1993; Ophir et al. 2008), it seems plausible that the social identity of females is only biologically meaningful after a pair bond has been formed, and possibly after a pair has reproduced. In the laboratory, for example, male prairie voles show territorial aggression (Pitkow et al. 2001; Young & Wang 2004; Gobrogge et al. 2009) and partner preferences (Gavish et al. 1983; Williams et al. 1992; Insel et al. 1995) only after a pair bond has been established. Social recognition may share similarities with these other important behaviours that mark an important life-history transition. Indeed, testing animals under experimental conditions with different motivational states (e.g. using females that are sexually receptive or unreceptive or testing males that are in different life-stages, such as being single or pair bonded) may have the potential to reveal whether males are unable or uninterested in investigating unfamiliar females. Preliminary data from our laboratory indicate that pair-bonded males do demonstrate robust social recognition of females (T. D. Blocker & A. G. Ophir, unpublished data), suggesting that pair bonding may facilitate the expression of social recognition for females by male prairie voles. This should come as no surprise since social recognition (and discrimination among females) is necessary for males to demonstrate the well-documented partner preferences that are characteristic of this species (Carter et al. 1995; Insel et al. 1995; Insel & Young 2001; Young & Wang 2004).

Environmental Context-dependent Social Recognition

Although social recognition appears to be context dependent based on the sex of the stimulus animal, the territorial context appears to have less effect on this form of learning and memory. Our results show that the ability of males to recognize other males is maintained, regardless of whether social interactions occur in a context containing olfactory cues that are familiar (home cage), unfamiliar (intruding in the home cage of another male) or neutral (clean bedding). In all contexts, males demonstrated behaviour consistent with social recognition of male conspecifics. However, social recognition was strongest when males were tested in their home cages, moderately strong when they were tested in the presence of an unfamiliar social cue, and relatively weak when they were tested in a context lacking social context. The unfamiliarity or absence of social context (i.e. social odour) increased the variation in social recognition among individuals of each group.

We originally expected that the local context of whether a male is in familiar or unfamiliar territory should affect his ability to recognize other males. This hypothesis was based on the idea that learning the social landscape should be more biologically meaningful for males when social interactions occur either within a home cage context (simulating a learning opportunity when focal males are defending a territory from intruders) or on the unfamiliar territory of another male (simulating a learning opportunity when focal males are acting as the intruder). In either case, we expected that the opportunity to learn social identity would be enhanced by the presence of a social cue (the smell of either self or another). Although our results did not strongly support either interpretation, the degree of individual variation was notably higher when the bedding was clean. The individual variation in the neutral context and the lack of a difference from zero in the recognition score suggests that either familiar social cues might facilitate the acquisition of social identity and discrimination between conspecifics, or absence of social cues may impair social recognition.

From a practical standpoint, this result serves as a warning to experimenters that conduct social recognition tests in clean bedding. While many laboratories perform social recognition tests in home cages (e.g. Ferguson et al. 2002; Choleris et al. 2012), it is common to use socially neutral cages to test social recognition (e.g. Sanchez-Andrade & Kendrick 2011). As in our experiment (Fig. 5), these studies often successfully demonstrate social recognition. However, our results also indicated that the overall group effects for recognition in non-home-cage conditions was greatly reduced, largely due to the aforementioned increase in individual variation within some groups (Fig. 6). We hypothesize that the lack of familiar social odours interferes with the ability of some animals to learn social identity. This hypothesis is consistent with Engelmann et al. (2011), who found that recognition in mice was impaired when tested in novel contexts but not when tested in familiar conditions. It is unclear whether this disruption is due to individual variation in stress responses (cf. Koolhaas et al. 2010), the natural capacity to recognize and discriminate between conspecifics, or something else. Testing recognition in a neutral context may reveal subtle variation in social behaviour or cognitive capacity, not easily detected when overall group effects have the potential to mask fundamental and functional individual variation. With this in mind, neutral contexts might be used deliberately if one were interested in understanding the natural variation within a population, which may be best studied under the context of behavioural syndromes or animal personality (Sih et al. 2004; Korte et al. 2005) rather than as group effects.

Our results may be best understood by considering the potential functional significance of social recognition. For example, it is unlikely that animals in nature would be faced with the challenge of learning the identity of neighbours in the absence of social cues (either of themselves or others). This disconnect may explain, to some extent, why the neutral context appeared to elevate the individual variation for social recognition. Alternatively, males may benefit from learning social identity when trespassing into home ranges, because learning the identity of neighbours when in the neighbour's territory might aid in evaluating the larger social landscape, either increasing extrapair mating opportunities or reducing the probability of potentially damaging agonistic interactions. To some extent, our results are consistent with this hypothesis, even though social recognition in the unfamiliar context was moderate when compared to the degree of recognition that took place in home cages. Finally, male prairie voles may be best at learning and distinguishing between male conspecifics when on familiar territory because knowing who your local competitors are would be important for successfully defending a home range. In other words, neighbouring males that intrude should be viewed as important threats worth remembering. The robust evidence for social recognition in the home cage context compared to relatively weaker group effects of social recognition in the other contexts may indicate that social recognition for some individuals may be enhanced by something akin to a 'home field advantage'. In California mice, *Peromyscus californicus*, winning a contest in a home territory increases the likelihood that an animal will win future bouts, but this advantage dissipates in unfamiliar territory even if the subject has won a contest (Fuxjager et al. 2010). This effect has also been described in other species, including birds (Krebs 1982), lizards (Olsson & Shine 2000), insects (Waage 1988; Kemp & Wiklund 2004), fish (Beaugrand et al. 1996) and humans (Carre et al. 2006). Our results might indicate that in addition to aggression, home territory could enhance cognitive performance in the form of better social memory.

Conclusion

Taken together, our results are consistent with the hypothesis that the social recognition abilities of male prairie voles are context dependent. Our intention was to use information from behavioural ecology to generate hypotheses about social recognition that we could test in the laboratory. By doing so, we hope to understand laboratory behaviour in a way that

accurately reflects the actual natural behaviour of the study species. Furthermore, because we studied contexts that are biologically meaningful, our results can be used to begin to bridge the gap between laboratory experiments on social cognition and place them into an evolutionary framework in which the behavioural ecology of the study species provides insight into the function of the behaviours studied. As understanding of the neural and hormonal mechanisms of such memory is improved (Engelmann & Landgraf 1994; Bielsky et al. 2004, 2005; Choleris et al. 2007; Clipperton-Allen et al. 2012; Lieberwirth et al. 2012), the opportunity to appreciate how social memory functions becomes increasingly accessible when it is paired with an understanding of the evolutionary context in which those mechanisms evolved.

Acknowledgments

We thank Britta Larsson for her help running trials. This work was supported by funding from the National Science Foundation to D.J.Z. (GRFP), the Oklahoma State University Wentz Research Scholars Award to L.F., and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development to A.G.O. (HD065604–01).

References

- Albers HE. The regulation of social recognition, social communication and aggression: vasopressin in the social behavior neural network. *Hormones and Behavior*. 2012; 61:283–292. [PubMed: 22079778]
- ASAB/ABS. Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*. 2012; 83:301–309.
- Bateman AJ. Intra-sexual selection in *Drosophila*. *Heredity*. 1948; 2:349–368. [PubMed: 18103134]
- Beaugrand JP, Payette D, Goulet C. Conflict outcome in male green swordtail fish dyads (*Xiphophorus helleri*): interaction of body size, prior dominance/subordination experience, and prior residency. *Behaviour*. 1996; 133:303–319.
- Bielsky IF, Hu SB, Ren X, Terwilliger EF, Young LJ. The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study. *Neuron*. 2005; 47:503–513. [PubMed: 16102534]
- 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; 29:483–493. [PubMed: 14647484]
- Bierbach D, Girndt A, Hamfler S, Klein M, Mucksch F, Penshorn M, Schwinn M, Zimmer C, Schlupp I, Streit B, et al. Male fish use prior knowledge about rivals to adjust their mate choice. *Biology Letters*. 2011; 7:349–351. [PubMed: 21208944]
- Bos N, d'Ettorre P. Recognition of social identity in ants. *Frontiers in Psychology*. 2012; 3:83. [PubMed: 22461777]
- Bychowski ME, Auger CJ. Progesterone impairs social recognition in male rats. *Hormones and Behavior*. 2012; 61:598–604. [PubMed: 22366506]
- Carre J, Muir C, Belanger J, Putnam SK. Pre-competition hormonal and psychological levels of elite hockey players: relationship to the 'home advantage'. *Physiology & Behavior*. 2006; 89:392–398. [PubMed: 16934844]
- Carter CS, DeVries AC, Getz LL. Physiological substrates of mammalian monogamy: the prairie vole model. *Neuroscience and Biobehavioral Reviews*. 1995; 19:303–314. [PubMed: 7630584]
- Carter CS, Getz LL. Monogamy and the prairie vole. *Scientific American*. 1993; 268:100–106. [PubMed: 8516669]
- Carter CS, Getz LL, Gavish L, McDermott JL, Arnold P. Male related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*). *Biology of Reproduction*. 1980; 23:1038–1045. [PubMed: 7008851]
- Choleris E, Clipperton-Allen AE, Phan A, Kavaliers M. Neuroendocrinology of social information processing in rats and mice. *Frontiers in Neuroendocrinology*. 2009; 30:442–459. [PubMed: 19442683]

- Choleris E, Little SR, Mong JA, Puram SV, Langer R, Pfaff DW. Microparticle based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. *Proceedings of the National Academy of Sciences, USA*. 2007; 104:4670–4675.
- Choleris E, Clipperton-Allen AE, Phan A, Valsecchi P, Kavaliers M. Estrogenic involvement in social learning, social recognition and pathogen avoidance. *Frontiers in Neuroendocrinology*. 2012; 33:140–159. [PubMed: 22369749]
- Clipperton-Allen AE, Lee AW, Reyes A, Devidze N, Phan A, Pfaff DW, Choleris E. Oxytocin, vasopressin and estrogen receptor gene expression in relation to social recognition in female mice. *Physiology & Behavior*. 2012; 105:915–924. [PubMed: 22079582]
- Clutton-Brock TH, Vincent ACJ. Sexual selection and the potential reproductive rates of males and females. *Nature*. 1991; 351:58–60. [PubMed: 2027382]
- Curtis JT. Does fertility trump monogamy? *Animal Behaviour*. 2010; 80:319–328. [PubMed: 20823948]
- delBarco-Trillo J, McPhee ME, Johnston RE. Nonagonistic familiarity decreases aggression in male Turkish hamsters, *Mesocricetus brandti*. *Animal Behaviour*. 2009; 77:389–393. [PubMed: 20126280]
- Dluzen DE, Ramirez VD, Carter CS, Getz LL. Male vole urine changes luteinizing-hormone-releasing hormone and norepinephrine in female olfactory-bulb. *Science*. 1981; 212:573–575. [PubMed: 7010608]
- Emlen ST, Oring LW. Ecology, sexual selection, and evolution of mating systems. *Science*. 1977; 197:215–223. [PubMed: 327542]
- Engelmann M, Landgraf R. Microdialysis administration of vasopressin into the septum improves social recognition in Brattleboro rats. *Physiology & Behavior*. 1994; 55:145–149. [PubMed: 8140159]
- Engelmann M, Wotjak CT, Landgraf R. Social discrimination procedure: an alternative method to investigate juvenile recognition abilities in rats. *Physiology & Behavior*. 1995; 58:315–321. [PubMed: 7568435]
- Engelmann M, Hadicke J, Noack J. Testing declarative memory in laboratory rats and mice using the nonconditioned social discrimination procedure. *Nature Protocols*. 2011; 6:1152–1162.
- Ferguson JN, Young LJ, Insel TR. The neuroendocrine basis of social recognition. *Frontiers in Neuroendocrinology*. 2002; 23:200–224. [PubMed: 11950245]
- Ferkin MH. Odor-related behavior and cognition in meadow voles, *Microtus pennsylvanicus* (Arvicolidae, Rodentia). *Folia Zoologica*. 2011; 60:262–276.
- Ferkin MH, Johnston RE. Meadow voles, *Microtus pennsylvanicus*, use multiple sources of scent for sex recognition. *Animal Behaviour*. 1995; 49:37–44.
- Ferkin MH, Combs A, delBarco-Trillo J, Pierce AA, Franklin S. Meadow voles, *Microtus pennsylvanicus*, have the capacity to recall the ‘what’, ‘where’, and ‘when’ of a single past event. *Animal Cognition*. 2008; 11:147–159. [PubMed: 17653778]
- Fisher, JB. Evolution and bird sociality. In: Huxley, J.; Hardy, AC.; Ford, EB., editors. *Evolution as a Process*. London: Allen & Unwin; 1954. p. 71–83.
- Fuxjager MJ, Forbes-Lorman RM, Coss DJ, Auger CJ, Auger AP, Marler CA. Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression. *Proceedings of the National Academy of Sciences, USA*. 2010; 107:12393–12398.
- Gaulin SJC, FitzGerald RW. Sexual selection for spatial-learning ability. *Animal Behaviour*. 1989; 37:322–331.
- Gavish L, Carter CS, Getz LL. Further evidences for monogamy in the prairie vole. *Animal Behaviour*. 1981; 29:955–957.
- Gavish L, Carter CS, Getz LL. Male–female interactions in prairie voles. *Animal Behaviour*. 1983; 31:511–517.
- Getz LL, Hofmann JE. Social organization in free-living prairie voles, *Microtus ochrogaster*. *Behavioral Ecology and Sociobiology*. 1986; 18:275–282.
- Getz LL, McGuire B. A comparison of living singly and in male–female pairs in the prairie vole, *Microtus ochrogaster*. *Ethology*. 1993; 94:265–278.

- Getz LL, McGuire B, Pizzuto T, Hofmann J, Frase B. Social organization of the prairie vole (*Microtus ochrogaster*). *Journal of Mammalogy*. 1993; 74:44–58.
- Getz LL, Simms L, McGuire B, Snarks M. Factors affecting life expectancy of the prairie vole, *Microtus ochrogaster*. *Oikos*. 1997; 80:362–370.
- Getz LL, McGuire B, Pizzuto TM. Does mate choice take place in free-living prairie voles, *Microtus ochrogaster*? Evidence from field data. *Acta Zoologica Sinica*. 2004; 50:527–534.
- Gheusi G, Bluthé RM, Goodall G, Dantzer R. Social and individual recognition in rodents: methodological aspects and neurobiological bases. *Behavioural Processes*. 1994; 33:59–87.
- Gobrogge KL, Liu Y, Young LJ, Wang ZX. Anterior hypothalamic vasopressin regulates pair-bonding and drug-induced aggression in a monogamous rodent. *Proceedings of the National Academy of Sciences, USA*. 2009; 106:19144–19149.
- Halpin ZT. Individual differences in the biological odors of the Mongolian gerbil (*Meriones unguiculatus*). *Behavioral Biology*. 1974; 11:253–259. [PubMed: 4847527]
- Hurst JL, Hayden L, Kingston M, Luck R, Sorensen K. Response of the aboriginal house mouse, *Mus spretus* Lataste, to tunnels odoured by conspecifics. *Animal Behaviour*. 1994; 48:1219–1229.
- Insel TR, Young LJ. The neurobiology of attachment. *Nature Reviews Neuroscience*. 2001; 2:129–136.
- Insel TR, Preston S, Winslow JT. Mating in the monogamous male: behavioral consequences. *Physiology & Behavior*. 1995; 57:615–627. [PubMed: 7777594]
- Jacquot JJ, Solomon NG. Experimental manipulation of territory occupancy: effects on immigration of female prairie voles. *Journal of Mammalogy*. 2004; 85:1009–1014.
- Jarcho MR, Mendoza SP, Mason WA, Yang X, Bales KL. Intranasal vasopressin affects pair bonding and peripheral gene expression in male *Callicebus cupreus*. *Genes, Brain and Behavior*. 2011; 10:375–383.
- Johnston RE. Memory for individual scent in hamsters (*Mesocricetus auratus*) as assessed by habituation methods. *Journal of Comparative Psychology*. 1993; 107:201–207. [PubMed: 8370274]
- Jones CM, Braithwaite VA, Healy SD. The evolution of sex differences in spatial ability. *Behavioral Neuroscience*. 2003; 117:403–411. [PubMed: 12802870]
- Kavaliers M, Colwell DD. Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proceedings of the Royal Society of London, Series B*. 1995; 261:31–35. [PubMed: 7644547]
- Kavaliers M, Choleris E, Agmo A, Pfaff DW. Olfactory-mediated parasite recognition and avoidance: linking genes to behavior. *Hormones and Behavior*. 2004; 46:272–283.
- Kemp DJ, Wiklund C. Residency effects in animal contests. *Proceedings of the Royal Society of London, Series B*. 2004; 271:1707–1711. [PubMed: 15306291]
- Kleiman DG. Monogamy in mammals. *Quarterly Review of Biology*. 1977; 52:39–69. [PubMed: 857268]
- Koolhaas JM, de Boer SF, Coppens CM, Buwalda B. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*. 2010; 31:307–321. [PubMed: 20382177]
- Korte SM, Koolhaas JM, Wingfield JC, McEwen BS. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the tradeoffs in health and disease. *Neuroscience and Biobehavioral Reviews*. 2005; 29:3–38. [PubMed: 15652252]
- Krebs JR. Territorial defence in the great tit (*Parus major*): do residents always win? *Behavioral Ecology and Sociobiology*. 1982; 11:185–194.
- Lieberwirth C, Liu Y, Jia X, Wang Z. Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. *Hormones and Behavior*. 2012; 66:357–366. [PubMed: 22465453]
- Macbeth AH, Edds JS, Young WS. Housing conditions and stimulus females: a robust social discrimination task for studying male rodent social recognition. *Nature Protocols*. 2009a; 4:1574–1581.

- Macbeth AH, Lee HJ, Edds J, Young WS. Oxytocin and the oxytocin receptor underlie intraspecific, but not interstrain, social recognition. *Genes, Brain and Behavior*. 2009b; 8:558–567.
- McGuire B, Getz LL. Alternative male reproductive tactics in a natural population of prairie voles *Microtus ochrogaster*. *Acta Theriologica*. 2010; 55:261–270.
- McGuire B, Pizzuto T, Getz LL. Potential for social interaction in a natural population of prairie voles, *Microtus ochrogaster*. *Canadian Journal of Zoology*. 1990; 68:391–398.
- McGuire B, Getz LL, Bemis WE, Oli MK. Social dynamics and dispersal in free-living prairie voles (*Microtus ochrogaster*). *Journal of Mammalogy*. 2013; 94:40–49.
- Mateo JM. Recognition systems and biological organization: the perception component of social recognition. *Annales Zoologici Fennici*. 2004; 41:729–745.
- Newman KS, Halpin ZT. Individual odours and mate discrimination in the prairie vole (*Microtus ochrogaster*). *Animal Behaviour*. 1988; 36:1779–1787.
- Olsson M, Shine R. Ownership influences the outcome of male–male contests in the scincid lizard, *Niveoscincus microlepidotus*. *Behavioral Ecology*. 2000; 11:587–590.
- Ophir AG, Phelps SM, Sorin AB, Wolff JO. Social but not genetic monogamy is associated with greater breeding success in prairie voles. *Animal Behaviour*. 2008; 75:1143–1154.
- Orians GH. On evolution of mating systems in birds and mammals. *American Naturalist*. 1969; 103:589–603.
- Ostfeld RS. The ecology of territoriality in small mammals. *Trends in Ecology & Evolution*. 1990; 5:411–415. [PubMed: 21232403]
- Pitkow LJ, Sharer CA, Ren X, Insel TR, Terwilliger EF, Young LJ. Facilitation of affiliation and pair-bond formation by vasopressin receptor gene transfer into the ventral forebrain of a monogamous vole. *Journal of Neuroscience*. 2001; 21:7392–7396. [PubMed: 11549749]
- Popik P, Van Ree JM. Neurohypophyseal peptides and social recognition in rats. *Progress in Brain Research*. 1998; 119:415–436. [PubMed: 10074804]
- Richmond, ME.; Stehn, RA. Olfaction and reproductive behavior in microtine rodents. In: Doty, RL., editor. *Mammalian Olfaction, Reproductive Processes and Behavior*. New York: Academic Press; 1976. p. 197–217.
- Sanchez-Andrade G, Kendrick KM. Roles of α - and β -estrogen receptors in mouse social recognition memory: effects of gender and the estrous cycle. *Hormones and Behavior*. 2011; 59:114–122.
- Sheehan MJ, Tibbetts EA. Specialized face learning is associated with individual recognition in paper wasps. *Science*. 2011; 334:1272–1275. [PubMed: 22144625]
- Sih A, Bell AM, Johnson JC, Ziemba RE. Behavioral syndromes: an integrative overview. *Quarterly Review of Biology*. 2004; 79:241–277. [PubMed: 15529965]
- Solomon NG, Jacquot JJ. Characteristics of resident and wandering prairie voles, *Microtus ochrogaster*. *Canadian Journal of Zoology*. 2002; 80:951–955.
- Solomon NG, Rumbaugh T. Odor preferences of weanling and mature male and female pine voles. *Journal of Chemical Ecology*. 1997; 23:2133–2143.
- Streatfeild CA, Mabry KE, Keane B, Crist TO, Solomon NG. Intraspecific variability in the social and genetic mating systems of prairie voles, *Microtus ochrogaster*. *Animal Behaviour*. 2011; 82:1387–1398.
- Tang-Martínez Z, Mueller LL, Taylor GT. Individual odours and mating success in the golden hamster, *Mesocricetus auratus*. *Animal Behaviour*. 1993; 45:1141–1151.
- Tate AJ, Fischer H, Leigh AE, Kendrick KM. Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philosophical Transactions of the Royal Society B*. 2006; 361:2155–2172.
- Temeles EJ. The role of neighbours in territorial systems: when are they ‘dear enemies’? *Animal Behaviour*. 1994; 47:339–350.
- Thor DH, Holloway WR. Persistence of social investigatory behavior in the male rat: evidence for long-term memory of initial copulatory experience. *Animal Learning & Behavior*. 1981; 9:561–565.
- Waage JK. Confusion over residency and the escalation of damselfly territorial disputes. *Animal Behaviour*. 1988; 36:586–595.

- Wacker DW, Ludwig M. Vasopressin, oxytocin, and social odor recognition. *Hormones and Behavior*. 2012; 61:259–265. [PubMed: 21920364]
- Williams JR, Catania KC, Carter CS. Development of partner preference in female prairie voles (*Microtus ochrogaster*): the role of social and sexual experience. *Hormones and Behavior*. 1992; 26:339–349. [PubMed: 1398553]
- Winslow JT, Camacho F. Cholinergic modulation of a decrement in social-investigation following repeated contacts between mice. *Psychopharmacology*. 1995; 121:164–172. [PubMed: 8545521]
- Wolff JO, Macdonald DW. Promiscuous females protect their offspring. *Trends in Ecology & Evolution*. 2004; 19:127–134. [PubMed: 16701243]
- Ydenberg RC, Giraldeau LA, Falls JB. Neighbors, strangers, and the asymmetric war of attrition. *Animal Behaviour*. 1988; 36:343–347.
- Young LJ, Wang ZX. The neurobiology of pair bonding. *Nature Neuroscience*. 2004; 7:1048–1054.
- Zala SM, Potts WK, Penn DJ. Scent-marking displays provide honest signals of health and infection. *Behavioral Ecology*. 2004; 15:338–344.

Highlights

- We tested whether male prairie voles can recognize conspecific individuals.
- Single males learned to recognize other males but not females.
- Social recognition did not differ across different territorial contexts.
- Territorial context may increase individual variation in social recognition.
- Natural history may influence social cognition in a context-dependent fashion.

Figure 1.

Social recognition apparatus and design used for all habituation/dishabituation and social discrimination tests. (a) Overhead view of the apparatus set for the habituation/dishabituation test. The main chamber and presentation chamber (pc) were constructed entirely of transparent acrylic. Dashed lines represent the front of the presentation chamber outfitted with holes for olfactory contact. Thick solid lines represent removable acrylic transparent walls. (b) The apparatus from an angle to depict the angled roof, on which a mirror was placed to provide a top-down view from the side. (c, d) Expected patterns of social investigation for the habituation/dishabituation test and the social discrimination test, respectively. Bars represent expected time spent investigating a stimulus animal (black and white) in the first four presentations (P1–P4) and the novel stimulus animal (grey) in the final presentation (P5). In (c), we also present the experimental design, in which we repeatedly presented individual stimulus animals separated by intertrial intervals (iti); the social discrimination test was run in the same way except that two stimulus animals were presented at a time.

Figure 2.

Mean $\pm$ SE time (in seconds) that focal males spent (a) inspecting or (b) attempting to contact stimulus animals during the habituation test (P1–P3; nontransformed data) and the dishabituation test (P4–P5). $N = 19$ for each test.

Figure 3. Mean $\pm$ SE time (in seconds) that focal males spent (a) inspecting or (b) attempting to contact novel or familiar stimulus females during the final trial of the social discrimination test ($N=19$).

Figure 4.

Mean $\pm$ SE recognition scores for focal males in each test. MHD: male habituation/dishabituation; FHD: female habituation/dishabituation; FSD: female social discrimination. Recognition scores were calculated using the time spent investigating stimulus animals as follows: $(P5 - P4) / (P5 + P4)$. Different letters above bars denote significant differences between groups based on post hoc tests.

Figure 5.

Mean $\pm$ SE time (in seconds) that focal males spent inspecting stimulus males in familiar ($N = 12$), unfamiliar ($N = 13$) or neutral ($N = 12$) contexts during the habituation/dishabituation test. (a) Inspection time during the habituation phase (P1–P3) (nontransformed, left) and during the dishabituation phase (P4–P5) (right). (b) Recognition scores $((P5 - P4) / (P5 + P4))$ for focal males under different territorial contexts.

Figure 6. Individual variation in inspection of stimulus males in familiar (black), unfamiliar (white), or neutral (grey) contexts during the habituation/dishabituation test (see Table 1 for group means).

Table 1

Mean $\pm$ SD time (in seconds) that male prairie voles spent investigating stimulus animals in different social contexts during each presentation period (with ranges in parentheses)

Period	Familiar, N=12	Unfamiliar, N=13	Neutral, N=12
P1	83.7 $\pm$ 26.1 (48–137)	64.7 $\pm$ 40.9 (10–134)	77.8 $\pm$ 22.0 (39–117)
P2	48.5 $\pm$ 29.4 (0–106)	42.8 $\pm$ 26.6 (0–94)	66.2 $\pm$ 35.2 (13–122)
P3	26.7 $\pm$ 9.7 (10–44)	32.5 $\pm$ 17.9 (0–66)	47.3 $\pm$ 32.1 (3–113)
P4	25.9 $\pm$ 12.6 (16–61)	25.6 $\pm$ 15.7 (0–60)	42.5 $\pm$ 27.2 (0–85)
P5	59.6 $\pm$ 26.1 (14–96)	52.3 $\pm$ 34.3 (0–119)	57.5 $\pm$ 39.6 (0–117)

See Fig. 6 for individual values of all focal males.