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Self-referencing and recognition in meadow voles, microtus pennsylvanicus

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Self-Discrimination in Meadow Voles, *Microtus pennsylvanicus*

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Abstract

Particular features of the signaling characteristics of the scent marks of temperate zone, seasonally breeding mammals may reflect differences in their reproductive state and, hence, be variable. Consequently, an individual's perception of self may depend more on the condition independent than on the condition-dependent signaling characteristics of the scent marks. Yet, we do not know whether an individual responds to changes in the signaling characteristics of its own scent marks, such as those associated with changes in an individual's reproductive state. Such changes may affect how and where an animal scent marks. Here we report on a series of experiments designed to test the hypothesis that individual meadow voles, *Microtus pennsylvanicus*, distinguish between scent marks they deposited when they were in different reproductive states. Results showed that voles discriminated their own scent marks from those of unfamiliar, same-sex conspecifics, and the scent marks of siblings. Voles did not behave as if they could distinguish between their own scent marks if the marks were deposited when the voles were in the same reproductive state, although the two scent marks used as stimuli differed in age by 30 d. However, they did so distinguish if they were exposed to scent marks taken when they were in different reproductive states. Overall, these findings suggest that voles behave as if their novel and familiar scent marks shared the similar signaling features. If, however, the reproductive condition of the voles differed when it provided the two scent marks, they behaved as if their own scent marks had different signal characteristics, which may have induced voles to treat the two scent marks as not being the same or having been deposited by two different donors. We speculate that the scent marks of individuals may have unique signaling characteristics that may be associated with that individual's 'current template for self'.

Introduction

Animals in a variety of taxa distinguish between the chemical cues and scent marks from those of familiar and unfamiliar conspecifics (Gosling & Roberts 2001; Mateo 2002, 2004; Johnston 2003; Todrank & Heth 2003; Thom & Hurst 2004; Holmes & Mateo 2007). Some animals also can discriminate between their own scent marks and chemical cues and those of conspecifics (Alberts 1992a; Bekoff 2001, 2002; Hernández et al. 2006; but see Palphramand & White 2007). Less is known, however, about how animals discriminate and respond to their own chemical cues and scent marks that may have been deposited at different times or when the animals were in a different condition, which may be affected by the reproductive status, diet, or social experiences of the donor (McClintock 2002; Leonard et al. 2005;

Pierce et al. 2007; Roberts 2007). Thus, a scent donor's condition may have changed since it last deposited scent marks in a particular area. Changes in the condition-dependent component of the signaling characteristics of an individual's scent mark (Roberts 2007) may be sufficient to alter its perception of self, and where and how it deposits its scent marks (Gosling 1982; Alberts 1992a; Gosling & Roberts 2001). If features of the signaling characteristic of its scent marks do not match the animal's current condition, they would need to mark again. These new markings would serve to provide current information about their state to indicate their presence and how recently they were in an area, and to distinguish their possessions or resources from those of others (Gosling 1982; Alberts 1992a,b).

It is implicit to this argument, that animals recall and discriminate between these scent marks at some later time (Beauchamp & Wellington 1984; Johnston 1993; Mateo & Johnston 2000a,b), and that the signaling features or some intrinsic property of the animals' scent marks are somewhat stable and distinct (Gosling & Roberts 2001). Two intrinsic components comprise the signaling features of the animals' scent marks, the condition-dependent component, which is labile, and the condition-independent component, such as the individual's major histocompatibility complex (MHC) or features of its major urinary proteins (MUP) (Gosling & Roberts 2001; Hurst et al. 2001; Roberts 2007). Thus, the condition-dependent component of the signaling features of the animals' scent marks may change as the state of the animal changes over time. For example, the attractiveness and responsiveness of voles to the scent marks of conspecifics depends on many factors such as diet and reproductive state, which are mediated by gonadal hormone titers (Ferkin & Johnston 1993; McClintock 2002; Prendergast et al. 2002). Thus, changes in circulating titers of gonadal steroids may affect the condition-dependent component of the signaling feature of a vole's scent mark, and how an individual responds to its own scent marks.

In the present study, we determined if individual voles behave as if they can distinguish among their own scent marks, those of same-sex siblings, and those of unfamiliar same-sex conspecifics. To make this determination, we used a habituation–dishabituation technique to test two hypotheses (Johnston 1993; Todrank et al. 1998; Ferkin et al. 1999; Mateo & Johnston 2000a). The first hypothesis is that an individual distinguishes between scent marks it deposited when it was in different reproductive states. Thus, voles would habituate to their present scent marks and spend less time investigating (sniffing and licking) such marks, but would spend more time investigating scent marks made when they were in a different condition. Such a response would indicate that the condition-dependent component may be more salient for self-discrimination than the condition-independent component of the signaling feature of a vole's scent mark. The alternate hypothesis is that an individual behaves as if it cannot distinguish between scent marks that were deposited when it was in two different reproductive states. Thus, voles would habituate to their present scent marks and spend less time investigating such marks, but would also spend similar amounts of time investigating scent marks taken when they were in each reproductive condition. Such a response would indicate that condition-independent component may be more salient for self-discrimination than the condition-dependent component of the signaling feature of a vole's scent mark. Before we tested the hypotheses, we first determined whether meadow voles discriminate between their own scent marks and those of unfamiliar conspecifics. Next, we assessed whether meadow voles discriminate between their own scent marks and those of siblings, and discriminate between their sibling's scent marks and those of unfamiliar conspecifics. This allowed us to determine if voles discriminate their own features of scent marks from similar features of scent marks of closely related individuals. Finally, we determined whether voles discriminate between their own scent marks, which may be a necessary feature of self-recognition (Gallup 1998; Sherman 1991; Alberts 1992a; Hauber & Sherman 2001, 2003; Bekoff & Sherman 2004; Morin 2006).

Materials and Methods

Animals

Meadow voles used in these experiments were third- and fourth-generation wild-caught individuals captured in north central Kentucky, southern Ohio, and central New York, USA. Voles were born and raised under long photoperiod (14:10 h, L:D, lights on at 0700h CST). All voles were weaned at 18 d age, housed with littermates until 35 d of age, and thereafter housed singly in clear plastic cages (27 × 16.5 × 12.5 cm; l, w, h, respectively). Cages contained cotton nesting material, water, and food (Laboratory Rodent Diet # 5008; PMI, St. Louis, MO, USA). Cages containing single animals were cleaned weekly and the cotton nesting material was replaced every 14 d. We followed Animal Care Protocol 501,

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which was approved by the IACUC at the University of Memphis. We adhered to the 'Guidelines for the use of animals in research' as published in Animal Behaviour (1991, 41, 183–186) and the laws of the country where the research was conducted.

We used different voles in and between experiments. The voles were 120–180 d old and sexually experienced, having sired or delivered at least one litter. The voles were housed singly for at least 3 wk prior to the study. None of the female voles were pregnant or lactating at the time of the study. Female meadow voles are induced ovulators that do not undergo regular estrus cycles (Keller 1985), but under long photoperiod will readily mate with males (Meek & Lee 1993). All testing was conducted between 0900 and 1300 h.

Experimental Design

We tested animals using habituation/dishabituation tasks in which they were exposed to their own current urine scent marks and those of conspecifics. The habituation technique has been used in several studies concerned with odor-based discrimination tasks (Johnston 1993; Todrank et al. 1998; Ferkin et al. 1999; Mateo & Johnston 2000a; Ferkin & Li 2005). In this task, a vole is presented three successive 3-min exposures with a donor's scent mark and the amount of time that it spends investigating the scent mark is recorded for each presentation; this is the habituation phase (Johnston 1993, 2003). Then, the vole is presented with that scent mark and the scent mark from a novel donor and the amount of time that the vole spends investigating both scent marks is recorded; this is the test phase (Johnston 1993, 2003). There was a 1-min interval between each exposure during both the habituation and test phases of a trial.

Previous studies using voles has shown that investigation time decreases across the habituation phase, and increases when the animal investigates the novel scent mark during the test phase. It is assumed that such test space results suggest that the vole is behaving as if the scent marks are somehow different (Ferkin et al. 1999). If the investigation time does not increase during the test phase when the vole investigates the novel scent, it is assumed that the vole is behaving as if the two scent marks are similar (Johnston 1993, 2003). In that we are measuring spontaneous behavior, a lack of discrimination does not indicate the inability to discriminate.

Specifically, we first compared the amount of time subjects investigated a scent mark during the first and third (the last exposure) of the exposure phase. Second, we compared the amount of time subjects investigated the two scent marks during the test phase. Each set of paired comparisons was analyzed with paired t-tests (Johnston 1993). We considered the results to be significant if $p < 0.05$.

The Scent Marks

We created urine scent marks by applying, with a sterile cotton swab, a streak of urine from donor voles onto the scent portion of a clean glass microscope slide (7.75 × 2.5 cm; Ferkin et al. 1999; Ferkin & Li 2005). The resulting 1.0 × 0.2-cm streak (urine scent mark) was allowed to dry for 60 s before the slide was placed into the home cage of the subject. The slide was suspended by a clasp and wire hanger approximately 1 cm above the substrate and against the wall opposite the subject's nest. The total amount of time that the subjects investigated the mark from the scent donors was recorded by an observer blind to the identity of the scent donor. Subjects had to lick or sniff the scent mark on the slide for the data to be included in the analyses (see expt 1 next for details). Each slide was used only once and then discarded.

Urine was collected from voles by placing the voles in collecting chambers. Each chamber was 30 cm high and 10 cm in diameter; the floor of the chamber was constructed of fine mesh so that urine, but not feces, could pass through into a collecting dish that sat directly below the mesh floor. The collected urine was placed into individually labeled vials using a sterile pipette and stored at −20°C for either 6 h d or 30 d, depending on the experiment. The 30-d interval was selected as it would likely encompass changes in the condition of free-living voles that are affected by changes in diet, changes in social status, or reproductive state (Keller 1985; Takahashi 1990; Ferkin 2007; Pierce et al. 2007). The frozen urine was thawed at room temperature before its use in an experiment. Each collecting chamber was cleaned thoroughly with soapy water and ethanol after each use.

Experiment 1 – Can Voles Discriminate between their Own Urine Marks and those of Same-Sex Conspecifics?

Animals

In this experiment, the scent donors and the subjects were the same sex, and similar in age (within 30 d), and similar in weight (within 5 g, approximately

9–11% of their body weight). Urine was collected from donors once, when they were between 110 and 115 d of age. Testing began 6 h after the collection of the urine samples from the donors.

Habituation Phase

In this experiment, and in subsequent experiments, there were two habituation tasks. Subjects (n = 12 males and 12 females) were presented for three successive 3-min exposures with a different glass slide that contained their own urine mark (familiar scent mark) or subjects (n = 11 males and 11 females) were presented for three successive 3-min exposures with a different glass slide that contained the urine scent mark of a same-sex conspecific (familiar scent mark).

Test Phase

In this experiment and subsequent experiments, there were two tests/discrimination tasks that followed 1 min after the final (third) exposure during the habituation phase. In this experiment, subjects (n = 12 males and 12 females) were presented for 3 min to a glass slide that contained their own urine mark (familiar scent mark) and a glass slide that contained the scent mark of a same-sex conspecific (novel scent mark). Another group of subjects (n = 11 males and 11 females) were presented for 3 min to a glass slide that contained their own urine mark (novel scent mark) and a glass slide that contained the scent mark of a same-sex conspecific (familiar scent mark). These latter voles had been exposed to the scent of a conspecific for three successive exposures during the habituation phase.

Experiment 2 – Can Voles Discriminate between their Own Urine Marks and those of Same-Sex Siblings?

Animals

Animal husbandry and urine collection followed those described in expt 1 with this notable exception. In this experiment, the scent donors and subjects were same-sex siblings (littermates) that were similar in weight (within 5 g). Testing began 6 h after the collection of the urine from the donors.

Habituation Phase

Male (n = 12) and female (n = 12) voles underwent three successive 3-min exposures to different glass slides containing their own urine mark (familiar scent mark). A different group of subjects (n = 12 males and 12 females) underwent three successive 3-min exposures with glass slides that contained the urine scent mark of a same-sex sibling (familiar scent mark).

Test Phase

During the test phase, subjects (n = 12 males and 12 females) were presented for 3 min each to glass slides in which their own urine mark was the familiar scent mark and the scent mark of a same-sex sibling was the novel scent mark. The other group of subjects (n = 12 males and 12 females) were presented with glass slides in which their own urine mark was the novel scent mark and scent mark of a same-sex sibling was the familiar scent mark.

Experiment 3 – Can Gonadectomized Meadow Voles Discriminate between their Own Scent Marks after Gonadectomy (No Hormone Replacement) and those of a Gonadectomized Same-Sex Sibling?

Animals

Animal husbandry and urine collection followed those described in expt 2, with these notable exceptions. First, the scent donors and subjects were gonadectomized when they were between 80 and 85 d of age; they received no replacement hormone. Second, urine was collected 30 d after the individuals underwent gonadectomy. This interval after surgery is more than sufficient to allow for the clearance of residual hormones in the gonadectomized male and female voles and to lower testosterone titers in males to approximately 0.2 ng/ml and estradiol titers in females to approximately 79.7 pg/ml (Leonard et al. 2005; Pierce et al. 2007). Testing began 6 h after the collection of the second urine samples. Voles were tested when they were 110–115 d of age, 30 d after gonadectomy.

Gonadectomy with No Steroid Hormone Replacement

At 70–80 d of age, 20 male and 20 female voles were anesthetized with a ketamine-xylazine cocktail (4.5 mg ketamine HCl/ml; Fort Dodge Laboratories, Fort Dodge, IA, USA and 0.45 mg xylazine HCl/ml; Butler Company, Columbus, OH, USA) prior to

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gonadectomy via a midline incision (Leonard et al. 2005; Pierce et al. 2007). Concurrent to surgery, an empty Silastic® capsule implant (Dow Corning, Midland, MI; od 0.077 in, id 0.058 in) was implanted into 10 males and 10 females. This allowed us to create a group of gonadectomized voles that did not receive gonadal hormone replacement.

Habituation Phase

Male (n = 10) and female (n = 10) voles underwent three successive 3-min exposures to different glass slides containing their own scent mark after gonadectomy (familiar scent mark). A different group of subjects (n = 10 males and 10 females) underwent three successive 3-min exposures with glass slides that contained the scent mark of a gonadectomized same-sex sibling (familiar scent mark).

Test Phase

During the test phase, one group of subjects (n = 10 males and 10 females) were presented for 3 min to a glass slide in which their own scent mark after gonadectomy was the familiar scent mark and the scent mark of their same sex after gonadectomy was the novel scent mark. The other group of subjects (n = 10 males and 10 females) were presented with a glass slide in which their own scent mark after gonadectomy was the novel scent mark and scent mark of their same-sex sibling after gonadectomy was the familiar scent mark.

Experiment 4 – Can Gonadectomized Voles Not Given Hormone Replacement Discriminate between their own Scent Marks before Gonadectomy and their own Scent Marks after Gonadectomy?

Animals

Animal husbandry, gonadectomy, and urine collection followed those described in expt 3, with these notable exceptions. In this experiment, the scent donors and subjects were the same individuals (n = 20 males and 20 females). Urine was collected twice from these voles. First, urine was collected 1 h before the individuals (80–85 d old) underwent gonadectomy; they received no hormone replacement. The second urine collection took place 30 d after the individuals underwent gonadectomy. Animals were tested when they were between 110 and 115 d of age, 30 d after gonadectomy.

Habituation Phase

Male (n = 10) and female (n 0) voles underwent three successive 3-min exposures to different glass slides containing their own scent marks before gonadectomy (familiar scent mark). A different group of subjects (n = 10 males and 10 females) underwent three successive 3-min exposures with glass slides that contained their own scent marks after gonadectomy (familiar mark).

Test Phase

During the test phase, subjects (n = 10 males and 10 females) were presented for 3 min to a glass slide in which their own scent mark before gonadectomy was the familiar scent mark and their own scent mark after gonadectomy was the novel scent mark. The other group of subjects (n = 10 males and 10 females) were presented with a glass slide in which their own scent mark before gonadectomy was the novel scent mark and the scent mark after gonadectomy was the familiar scent mark.

Experiment 5 – Can Gonadectomized Voles Given Hormone Replacement Discriminate between their Own Scent Marks before Gonadectomy and their Own Scent Marks after Gonadectomy?

Animals

Animal husbandry, urine collection, gonadectomy, and onset of testing followed those described in expt 4, with this notable exception. In this experiment, gonadectomized voles received hormone replacement concomitant with gonadectomy when they were between 80 and 85 d of age. Voles were tested 30 d after gonadectomy and hormone replacement, when they were 110–115 d of age.

Steroid Hormone Replacement

The procedure follows that detailed for expt 4 except in the present experiment, scent donors were gonadectomized voles and given hormone replacement. Thus, concurrent with surgery, an identical capsule (Dow Corning; od 0.077 in, id 0.058 in) containing 15 mm of active length of testosterone was implanted subcutaneously in the interscapular region of the males (n = 20 males) and 5 mm of active length of the estradiol 17-β was implanted into females (n = 20 females; Sigma Chemical Co., St Louis, MO, USA; Leonard et al.

2005; Pierce et al. 2007). Capsules of this dimension and gonadal steroid hormone concentrations maintain circulating testosterone titers at 1.2 ± 0.2 ng/ml (males) and 290.4 ± 24.7 pg/ml (females), titers comparable with those prior to gonadectomy and similar to those of free-living meadow voles during the breeding season (Leonard et al. 2005; Pierce et al. 2007).

Habituation Phase

Male (n = 10) and female (n = 10) voles underwent three successive 3-min exposures to different glass slides containing their own scent marks before gonadectomy (familiar scent mark). A different group of subjects (n = 10 males and 10 females) underwent three successive 3-min exposures with glass slides that contained their own scent marks after gonadectomy (familiar scent mark).

Test Phase

During the test phase, subjects (n = 10 males and 10 females) were presented for 3 min to a glass slide in which their own scent mark before gonadectomy was the familiar scent mark and their own scent mark after gonadectomy was the novel scent mark. The other group of subjects (n = 10 males and 10 females) were presented with a glass slide in which their own scent mark before gonadectomy was the novel scent mark and the scent mark after gonadectomy was the familiar scent mark.

Results

Experiment 1 – Self vs. Unfamiliar, Same-Sex Conspecific

Voles habituated to their own scent marks [male subjects, $t\ (23) = 3.55$, $p < 0.001$ and female subjects, $t\ (23) = 3.73$, $p < 0.001$, Fig. 1a] and to the scent marks of unfamiliar same-sex conspecifics [male subjects, $t\ (21) = 3.47$, $p = 0.002$ and female subjects, $t\ (21) = 3.17$, $p = 0.004$, Fig. 1b]. Voles also behaved as if they could distinguish between their own scent marks and those of unfamiliar, same-sex conspecifics, independent of the order of presentation of these scent marks. Male voles spent more time during the test phase investigating the novel scent mark, the scent mark not offered during the habituation phase, than the familiar scent mark, the scent mark offered in both the habituation and the test phases [$t\ (21) = 3.22$, $p = 0.002$, Fig. 1a; $t\ (21) = 3.09$, $p = 0.005$, Fig. 1b]. Likewise, females subjects spent more time during the test phase investigating the novel scent mark than the familiar scent mark [$t\ (23) = 3.45$; $p = 0.002$, Fig. 1a; $t\ (23) = 3.27$, $p = 0.001$, Fig. 1b].

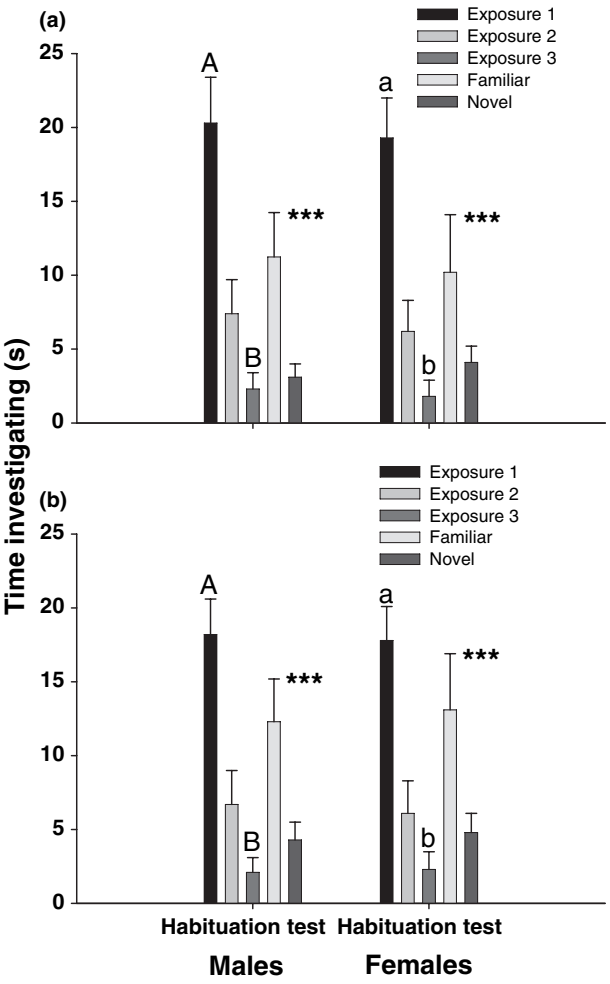

Fig. 1: The mean amount of time in seconds (±SE) that female and male voles spent investigating (sniffing and licking) either (a) the scent mark of an unfamiliar, same-sex conspecific during three successive 3-min exposures separated by a 1-min interval (the habituation phase) and a 3-min exposure to their own scent mark and the scent mark of an unfamiliar, same-sex conspecific (test phase) or (b) their own scent mark during three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to the scent mark of an unfamiliar, same-sex conspecific and their own scent mark (test phase). Histograms that represent exposures 1 and 3, which are capped with different letters (in the same case) are statistically different ($p < 0.05$); *** indicates that the test trials (novel vs. familiar scent mark) were statistically different ($p < 0.005$).

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Experiment 2 – Self vs. Same-Sex Sibling (Littermate)

Individuals habituated to their own scent marks [male subjects, t (23) = 4.09, p < 0.001 and female subjects, t (23) = 3.81, p < 0.001, Fig. 2a] and to those of a same-sex sibling [male subjects, t (23) = 3.76, p < 0.001 and female subjects, t (23) = 3.72, p < 0.001, Fig. 2b]. Voles also behaved as if they could distinguish between their own scent marks and those of their siblings, independent of the order of presentation of these scent marks. Male voles spent more time during the test phase investigating the novel scent mark than the familiar scent mark [t (23) = 2.59, p = 0.01, Fig 2a; t (23) = 3.10, p = 0.005, Fig. 2b]. Likewise, females subjects spent more time during the test phase investigating the novel scent mark than the familiar scent mark [t (23) = 2.58, p = 0.016, Fig. 2a; t (23) = 3.03, p = 0.006, Fig. 2b].

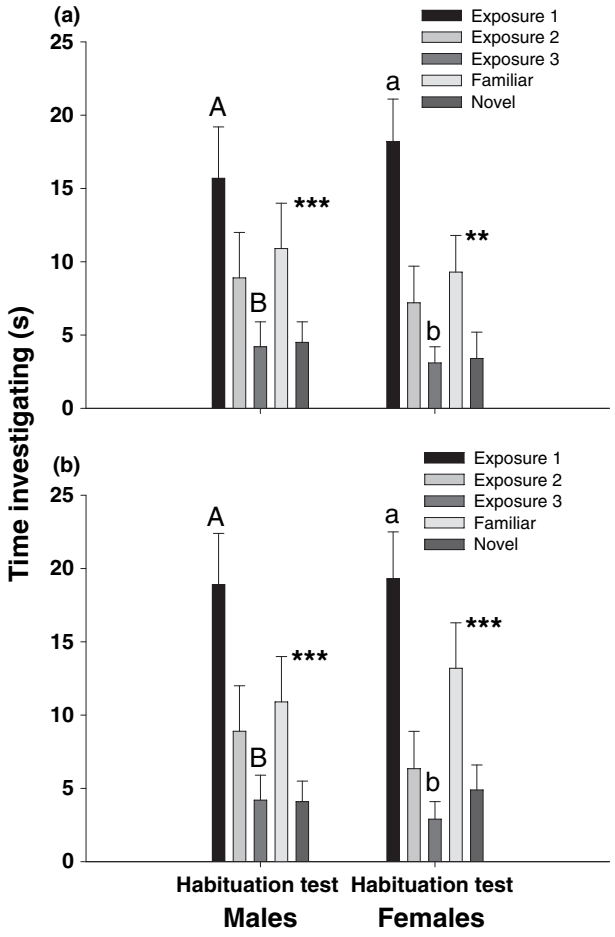

Fig. 2: The mean amount of time in seconds (±SE) that female and male voles spent investigating either (a) the scent mark of sibling during three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent mark and a same-sex sibling (littermate; test phase) or (b) their own scent mark during three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to the scent mark of same-sex sibling and their own scent mark (test phase). Histograms that represent exposures 1 and 3, which are capped with different letters (in the same case) are statistically different (p < 0.05); *** indicates that the test trials (novel vs. familiar scent mark) were statistically different (p < 0.005); ** indicates p < 0.01.

Experiment 3 – Current Self (After Gonadectomy; Gonadectomized + No Hormone Replacement) vs. Gonadectomized (No Hormone Replacement) Same-Sex Sibling

Voles habituated to their own scent marks [male subjects, t (19) = 2.41, p = 0.026 and female subjects, t (19) = 3.56, p = 0.002, Fig. 3a] and to those of a gonadectomized same-sex sibling [male subjects, t (19) = 3.04; p = 0.006 and female subjects, t (19) = 3.17, p = 0.005, Fig. 3b]. Taken together, gonadectomy did not alter a vole's ability to habituate to its scent marks and those of an unfamiliar, gonadectomized, same-sex sibling.

Voles discriminated between their own scent marks and those of their siblings, independent of the order of presentation of these scent marks. Males spent more time during the test phase investigating the novel scent mark than the familiar scent mark [t (19) = 2.34, p = 0.03, Fig. 3a; t (19) = 3.39, p = 0.003, Fig. 3b]. Females also spent more time investigating the novel scent mark than the familiar scent mark [t (19) = 2.67, p = 0.01, Fig. 3a; t (19) = 3.13, p = 0.005, Fig. 3b].

Experiment 4 – 'Current Self' (After Gonadectomy; Gonadectomized + No Hormone Replacement) vs. 'Past Self' (Before Gonadectomy; Intact Gonads)

Voles habituated to their scent marks after gonadectomy [male subjects, (t (19) = 2.28, p = 0.034 and female subjects, t (19) = 3.52, p = 0.002, Fig. 4a] and to their scent marks before gonadectomy [male subjects, t (19) = 3.41, p = 0.003 and female subjects, t (19) = 4.06, p < 0.001, Fig. 4b]. Thus, gonadectomy did not affect whether voles could habituate to their own scent marks.

Gonadectomy, however, affected whether voles could discriminate between their own scent marks. That is, voles behaved as if they could distinguish between their scent marks before gonadectomy (past self) and their scent marks after gonadectomy (current

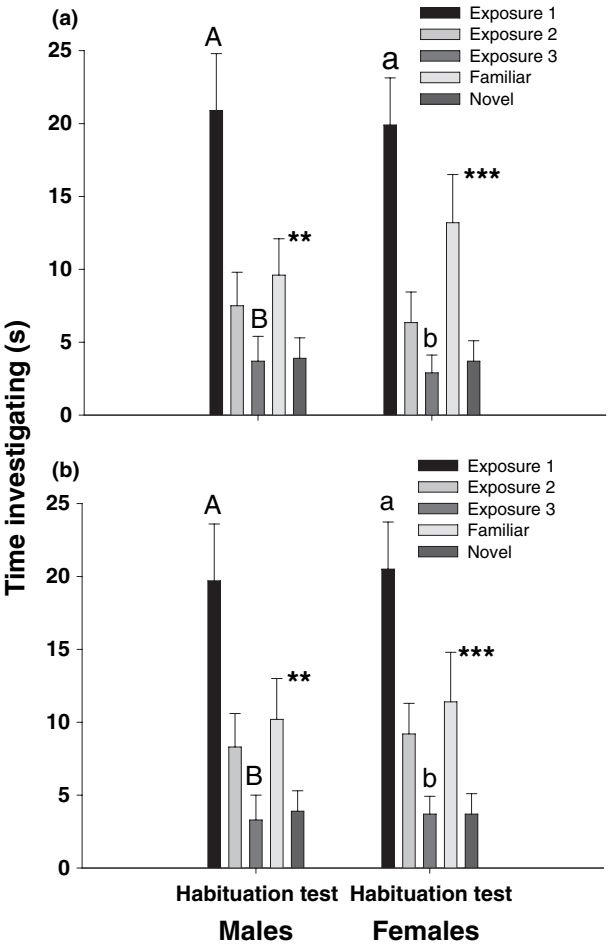

Fig. 3: The mean amount of time in seconds (±SE) that gonadectomized female and male voles spent investigating either (a) their own scent mark (after gonadectomy) during three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent mark (after gonadectomy) and the scent mark of a gonadectomized, same-sex sibling (littermate; test phase) or (b) the scent mark of a gonadectomized, same-sex sibling during three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent mark and the scent mark of a gonadectomized, same-sex sibling (test phase). Histograms that represent exposures 1 and 3, which are capped with different letters (in the same case) are statistically different ($p < 0.05$); *** indicates that the test trials (novel vs. familiar scent mark) were statistically different ($p < 0.005$); ** indicates $p < 0.01$.

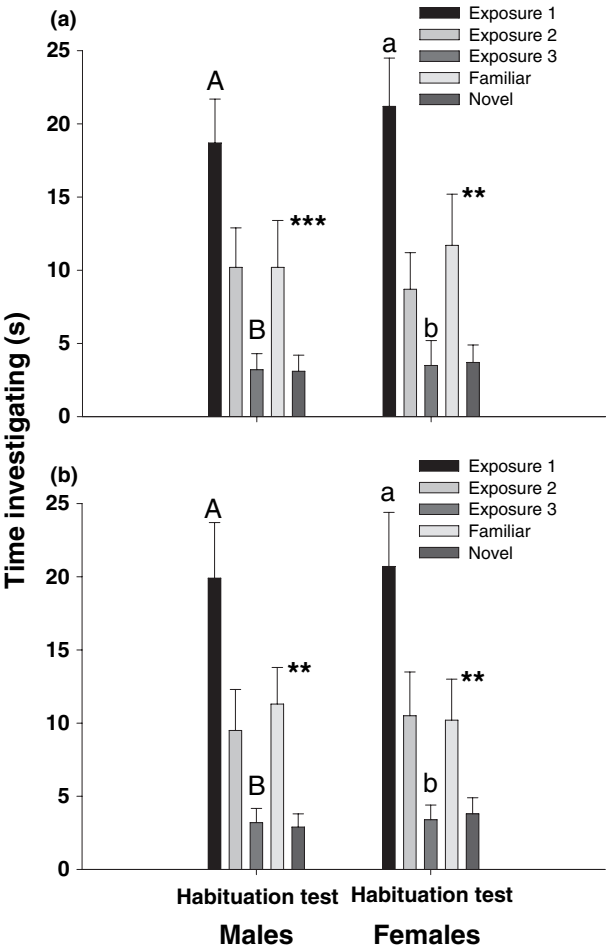

Fig. 4: The mean amount of time in seconds (±SE) that gonadectomized female and male voles spent investigating either (a) their own scent mark after gonadectomy on three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent marks (post-gonadectomy) and their own scent mark before gonadectomy (test phase) or (b) their own scent mark (before gonadectomy) on three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent marks after gonadectomy and their own scent mark before gonadectomy (test phase). Histograms that represent exposures 1 and 3, which are capped with different letters (in the same case) are statistically different ($p < 0.05$); *** indicates that the test trials (novel vs. familiar scent mark) were statistically different ($p < 0.005$); ** indicates $p < 0.01$.

self), independent of the order of presentation of these scent marks. Male voles spent more time during the test phase investigating the novel scent mark than the familiar scent mark [$t(19) = 3.71$, $p = 0.005$, Fig. 4a; $t(19) = 3.34$, $p = 0.003$, Fig. 4b]. Similarly, female voles spent more time investigating the novel scent than the familiar scent [$t(19) = 3.12$, $p = 0.005$, Fig. 4a; $t(19) = 3.29$, $p = 0.003$, Fig. 4b].

Experiment 5 – 'Current Self' (After Gonadectomy; Gonadectomized + Hormone Replacement) vs. 'Past Self' (Before Gonadectomy; Intact Gonads)

Voles habituated to their scent marks after gonadectomy [male subjects, $t(19) = 3.43$, $p = 0.002$ and female subjects, $t(19) = 3.26$, $p = 0.004$, Fig. 5a] and to their scent marks before gonadectomy [male subjects, $t(19) = 3.18$, $p = 0.005$ and female

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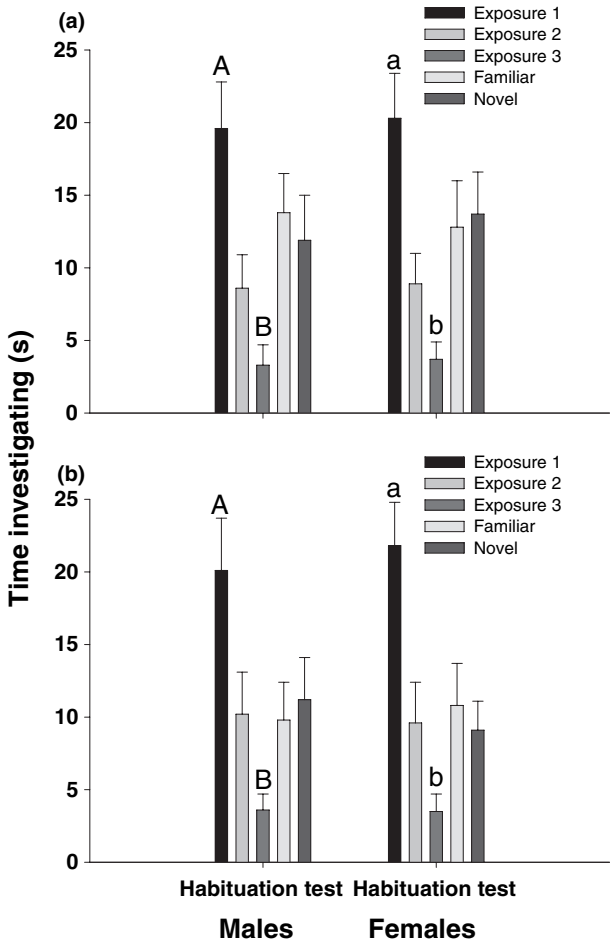

Fig. 5: The mean amount of time in seconds (±SE) that gonadectomized + hormone female and male voles spent investigating either (a) their own scent mark after gonadectomy on three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent marks (post-gonadectomy) and their own scent mark before gonadectomy (test phase) or (b) their own scent mark before gonadectomy on three successive 3-min exposures separated by a 1-min interval and a 3-min exposure to their own scent marks after gonadectomy and their own scent mark before gonadectomy (test phase). Histograms that represent exposures 1 and 3, which are capped with different letters (in the same case) are statistically different ($p < 0.05$); there was no significant difference ($p > 0.05$) in investigation time during the test phase.

subjects, $t\ (19) = 4.17$, $p < 0.001$, Fig. 5b]. Thus, gonadectomy and hormone replacement did not affect whether voles could habituate to their own scent marks.

Hormone replacement following gonadectomy affected whether voles could discriminate between their own scent marks. That is, regardless of the order of presentation of these scent marks, voles behaved as if they could *not* discriminate between their scent marks after gonadectomy (current self) and their scent marks before gonadectomy (past self). Regardless of the order of presentation of these scent marks, males spent similar amounts of time during the test phase investigating the novel scent mark and the familiar scent mark [$t\ (19) = 0.94$, $p = 0.35$, Fig. 5a; $t\ (19) = 0.33$, $p = 0.74$, Fig. 5b]. Likewise, females spent similar amounts of time during the test phase investigating the novel scent mark and the familiar scent mark [$t\ (19) = 1.11$, $p = 0.28$, Fig. 5a; $t\ (19) = 0.41$, $p = 0.68$, Fig. 5b].

Discussion

The present study addressed questions surrounding self-discrimination in meadow voles. We did so by devising five experiments that used the habituation–dishabituation paradigm (Todrank et al. 1998; Ferkin et al. 1999; Johnston 2003) to determine whether voles could behave as if they distinguish among their own scent marks, those of unfamiliar individuals, and familiar siblings. Our first experiment examined whether voles could distinguish their own scent marks from those of other same-sex conspecifics. During the exposure phase, voles were presented three times in succession to their own scent marks or those of a same-sex conspecific. The voles spent less time during each successive exposure investigating these scent marks, suggesting that the voles habituated to these 'familiar marks'. Voles, like other rodents, rapidly habituate to scent marks that they are exposed to repeatedly (Johnston 1993; Ferkin et al. 1999; Mateo & Johnston 2000a,b; Ferkin & Li 2005). During the test phase voles were exposed simultaneously to a familiar scent mark and a novel scent mark, a mark that they were not exposed to during the exposure phase. Voles spent more time investigating the novel scent mark than the familiar scent mark, supporting the notion that these scent marks may have different signal characteristics and come from different donors (Johnston 1993, 2003; Ferkin et al. 1999; Mateo 2002, 2004; Todrank & Heth 2003). These results show that voles, along with a variety of animals, behave as if they can distinguish between self and non-self using differences in the signaling features of the scent marks of the donors (Alberts 1992a; Bekoff 2001; Tsutsui 2004; Hernández et al. 2006; but see Palphramand & White 2007).

In expts 2 and 3, we determined if individual voles discriminate their own scent marks from those of the same-sex sibling. In both experiments, we found that after three successive presentations to

either their own scent marks or those of a same-sex sibling voles spent less time during each exposure investigating each scent mark. During the test phases of expts 2 and 3, voles spent more time investigating the novel scent mark than the scent mark they became familiar with during the habituation phase, suggesting that voles behaved as if they could distinguish between their own scent marks and those of the siblings. Similar results have been reported for gonadectomized house mice (Ferkin & Li 2005), suggesting that gonadectomized does not affect the ability for some rodents to habituate and later discriminate between their own scent marks and those of conspecifics. In addition, several studies have shown that individuals can identify close kin (Mateo & Johnston 2000a,b; Todrank & Heth 2003; Hernández et al. 2006; Holmes & Mateo 2007). When compared, the results of expts 1 and 2 suggest that the fixed-signaling characteristics and condition-dependent characteristics of scent marks (Gosling & Roberts 2001; Roberts 2007) provide sufficient detail for voles to act as if they can distinguish between their own scent marks and those belonging either to same-sex siblings or unfamiliar same-sex conspecifics. The results of expt 3, however, suggest that although the condition-dependent signaling characteristics of a vole's scent mark changed, gonadectomized voles behaved as if they could distinguish between their own pre-gonadectomy scent marks and those of their same-sex siblings. This result can be attributed to two different hypotheses. First, the condition-independent signaling component may have provided sufficient detail to allow voles to discriminate between their own scent mark and those of the same-sex conspecifics as it does in house mice (Hurst et al. 2001; Roberts 2007). Second, some aspect of the condition-dependent component of the scent marks was not altered enough by gonadectomy to prevent voles from acting as if they could distinguish between their own scent marks and those of the siblings.

In expts 4 and 5, we found that the reproductive condition of the individual voles did not affect whether they could habituate to scent marks, which is consistent with a previous study using gonadectomized mice as subjects (Ferkin & Li 2005). However, differences in the scent donor's reproductive state when its scent marks were collected affected how voles responded to their own scent marks during the test phase in expts 4 and 5. In expt 5, gonadectomized voles that had received hormone replacement spent similar amounts of time investigating the familiar scent mark, the mark they had been exposed to during the habituation phase, and the novel scent mark. That is, they behaved as if their previous (before gonadectomy) and current scent marks (after gonadectomy) had similar signaling characteristics. In contrast during expt 4, gonadectomized voles that received no hormone replacement behaved as if their previous (before gonadectomy) and current scent marks (after gonadectomy) had different signaling characteristics. During the test phase, these voles spent more time investigating the novel scent mark than the familiar scent mark. The difference in the response of voles in expts 4 and 5 to their own scent marks may lie in differences in the condition-dependent signaling characteristics of their scent marks. In expt 5, the gonadectomized individual had high titers of circulating gonadal steroid hormones because they received hormone replacement, when their scent marks were collected. In contrast, in expt 4, the scent marks were collected when the individual had high titers of circulating gonadal steroid hormones before gonadectomy and when that individual had low titers of gonadal steroids after gonadectomy and no hormone replacement. Thus in expt 4, but not expt 5, the condition-dependent signaling characteristic of the scent marked changed. This difference suggests that the fixed-signaling component did not provide sufficient detail to voles that were gonadectomized and received no hormone replacement to treat their scent marks before gonadectomy and after gonadectomy as being similar. If this conjecture is correct, and if the condition-dependent signaling feature of an individual's scent mark does not match its current template for self, the individual may renew or alter its olfactory-guided behaviors to compensate for such changes. This speculation is consistent with studies on voles and other mammals showing that the individuals alter their self-grooming and scent-marking behaviors as the reproductive state changes (González-Mariscal et al. 1990; Coquelin 1992; Ferkin et al. 1996; 2004; Leonard et al. 2005; Pierce et al. 2007).

For voles, the mechanism underlying discrimination of self from non-self may be complex. Perhaps, the signaling features of an individual's scent marks are associated with that individual's 'current template for self'. In this way, individuals compare their own scent marks and those of conspecifics to their unique template for self, and act accordingly (Reeve 1989; Tsutsui 2004; Holmes & Mateo 2007). This association between template for self and signaling characteristics of scent marks is not new and is akin to the hypothesized mechanisms for phenotype and

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scent matching (Gosling 1982; Hauber & Sherman 2001, 2003; Neff & Sherman 2002; Liebert & Starks 2004; Mateo 2004), and may have functional ramifications. By discriminating between their own marks and those of the same-sex conspecifics, individuals may be able to monitor the activity and movement of potential same-sex rivals in a given area or signal ownership or possession of a resource or territory (Alberts 1992a; Cooper et al. 1999; Bekoff & Sherman 2004; Palphramand & White 2007). In addition, individuals could use differences in their own scent marks as navigational guides to allow them to distinguish between areas they have visited recently from those they have not visited recently, re-fresh territorial borders or to re-mark their mates with fresh scents, or to signal up-to-date information about their reproductive state to nearby conspecifics (Gosling 1982; Sherman 1991; Alberts 1992a; Bekoff & Sherman 2004). The ability to discriminate among one's scent marks may be a feature associated with self-recognition (Gallup 1998; Bekoff & Sherman 2004; Morin 2006).

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